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THE AMERICAN
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THE EFFECT OF DIFFERENT PER CENTS OF PROTEIN IN THE DIET

V. The Offspring[1]

JAMES ROLLIN SLONAKER

From the Department of Physiology, Stanford University, California

Received for publication March 16, 1931

In the first paper of this series we gave in detail the plan and procedure of this experiment (Slonaker, 1931a). Five different groups of rats, composed of 18 pairs each and designated I, II, III, IV and V, were fed continuously on carefully prepared synthetic diets composed of the same ingredients and containing 10, 14, 18, 22 and 26 per cent of protein respectively. The experiment was carried through several generations and the offspring were continued on the same diet as that of the parents.

Since several of the later generations are still under observation this paper will deal only with offspring, which we have designated generation I, of the original matings. Several of the original 180 animals with which the experiment started died before attaining the average life span. When this occurred the survivor of the pair was remated. Generation I, therefore, includes not only the offspring of the original 18 pairs in each group, but also of such rematings as were required. The number of replacements in each of the groups from I to V for the males was 7, 5, 4, 5, 9 and for the females 6, 3, 3, 5, 8 respectively.

Young born. We showed in our paper dealing with reproduction (Slonaker, 1931b) that there were several of the original animals which proved to be sterile. The per cents of sterility in the females used were group I, none; II, 4; III, 19; IV, 39; and V, 50 per cent. In table 1 we have given the number of fertile females in each group which gave birth to the young

[1] This research has been assisted by the Department of Physiology, the National Research Council through its Committee for Research in Problems of Sex, the National Live Stock and Meat Board, the Fleischmann Company and their successors, the Standard Brands, Inc., and the coöperation of Albers Bros. Milling Company.

constituting generation I. This table shows that the total number of litters and young born in each group was greatest in group I and decreased as the per cent of protein in the diet increased. This would be expected as the number of fertile females followed the same order. But we also see that the average size of the litters decreased in the same manner from 5.34 in

TABLE 1

Giving the total number, the average number and size of the litters, and the number and average weight at birth of each sex in the five groups

GROUP	FERTILE FEMALES	LITTERS BORN			YOUNG BORN					
							Males		Females	
		Total number	Average number	Average size	Total number	Average number	Number	Average weight	Number	Average weight
I	23	122	5.30	5.34	652	28.3	324	5.21	328	5.17
II	21	121	5.76	5.15	623	29.6	312	5.27	311	5.05
III	17	67	3.94	4.68	314	18.5	141	5.50	173	5.34
IV	14	42	3.00	4.38	184	13.1	88	5.65	96	5.27
V	13	38	2.92	4.00	152	11.7	66	5.48	86	5.35

TABLE 2

Giving the number and average weight of each sex at weaning, the average total and daily gain while nursing in each group

Young weaned

GROUP	AVERAGE AGE	TOTAL NUMBER	MALES		FEMALES		WEIGHT GAINED					
							Males			Females		
							Total grams	Average daily gain		Total grams	Average daily gain	
			Number	Average weight	Number	Average weight		Grams	Per cent		Grams	Per cent
I	26	388	197	21.3	191	21.0	16.09	0.618	11.86	15.83	0.608	11.78
II	25	446	232	25.5	214	24.6	20.23	0.803	15.23	19.55	0.782	15.49
III	24	160	76	26.4	84	27.2	20.90	0.840	15.28	21.66	0.902	16.31
IV	26	111	56	29.6	55	27.4	23.95	0.920	16.29	23.13	0.850	16.15
V	28	75	32	29.4	43	33.6	23.92	0.854	15.57	28.25	1.000	18.69

group I to 4.00 in group V. When we consider the average number of litters and average number of young per fertile female we find that group II was most efficient and was followed by groups I, III, IV and V in order. This shows the efficiency of the diets on the reproduction of fertile females. But this does not take into consideration the sterile females. The true

values of the diets used is shown only when the average number of young borne by all the females used in each group is considered. This gave the following averages in the order of greatest to least efficient: group II, 29.6; group I, 27.2; group III, 14.9; group IV, 8; and group V, 5.8. The order of efficiency of the diets is not changed from that of fertile females. It shows that the diet containing 14 per cent protein was most effective, and that the 10 per cent protein diet was almost as good. These data also show that the greater the per cent of protein above 14 per cent, the more inhibitory were the effects on the number of litters and the number of young born. This inhibition may have caused fewer ova to have been liberated, a reduction in the vitality of the sperm, the ova, or both, or in greater prenatal mortality of the young. Possibly all three were instrumental in causing the reduction in the number of young born. Further investigation will be necessary to determine this.

TABLE 3

Giving the sex ratios at birth and at weaning and the per cent which died in generation I in each group

GROUP	YOUNG BORN					YOUNG WEANED					PER CENT DIED BEFORE WEANING		
	Total	Males	Females	Sex ratio		Total	Males	Females	Sex ratio		Males	Females	Total
				Males	Females				Males	Females			
I	652	324	328	99	100	388	197	191	103	100	39	42	40
II	623	312	311	100	100	446	232	214	108	100	26	31	28
III	314	141	173	81	100	160	76	84	90	100	46	51	49
IV	184	88	96	92	100	111	56	55	102	100	36	43	40
V	152	66	86	77	100	75	32	43	75	100	52	50	51

Table 1 also gives the number of each sex born in the different groups. It is seen that, with the exception of group II, there were more females born in each group than males. This suggests that the prenatal mortality of the males was greater than that of the females. The sex ratio of the young at birth in each group is given in table 3. It is seen that the ratio in each group was below the normal average of 106 males to 100 females. Group II, followed closely by group I, showed the nearest to normal and group V had the lowest sex ratio. These data show that as the per cent of protein in the diet increased above 14 per cent there was not only a corresponding reduction in the number of young born, but also that the prenatal development of the males was more affected than that of the females.

Young weaned. Mortality. In table 2 are given the total number of young and the number of each sex weaned together with the average age at which the litters were weaned in each of the groups. In our former

paper (Slonaker, 1931b) we gave the per cent of young which were nursed to the weaning age. These were for groups I to V 60, 72, 51, 60 and 49 per cent. In table 3 we have given the per cent of males, of females, and of total young born which died in each group before weaning. This shows that group II had the lowest mortality and that group V had the greatest of any of the groups. It also shows that, with the exception of group V, the mortality of the females during the nursing period was greater than that of the males. We found that the greatest mortality in all groups was during the nursing period and occurred usually within the first six or eight days after birth. The greater postnatal mortality of the females in this case corresponds with our previous results (Slonaker, 1928) and further indicates a greater vitality of the males than of the females during the nursing period. These data show that group II reared a larger number of young and that this number represented a higher per cent of the young born than in any of the other groups. They also show that the diet containing 14 per cent of protein was more efficient and produced more vigorous young than any of the other diets.

Cannibalism. It is well known that rats manifest cannibalism to a marked degree. The mothers often not only eat some or all of their young at birth or soon afterward, but also mature rats have often been found partly eaten by their cage mates. In a former paper (Slonaker, 1923) in seven different groups of rats comprising 2388 young the per cents of the 1216 which died that were eaten ranged from 65 to 85 per cent. In a later paper (Slonaker, 1928) we found the per cents of cannibalism of the dead were 90 and 95. In this research the per cents of those which died that were eaten in the different groups from I to V were 89, 85, 82, 95 and 88 respectively. Group III, followed closely by group II, showed the least manifestation of cannibalism, but the differences were not sufficiently great as to warrant any definite conclusions.

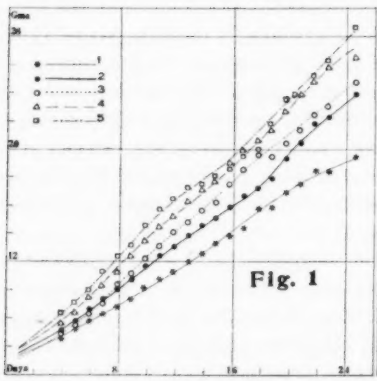
Growth. The average weight at birth of each sex in the different groups is given in table 1. Since it was impossible to get the weight of all the young immediately after birth many of these weights included those of young which had previously nursed. All the figures given, therefore, are slightly greater than the true birth weights. The same error, however, existed in all the groups and a comparison is permissible. As will be seen the males were heavier than the females in each group. It is also noted the groups which were fed the richer protein diets had in general the heaviest young. This may have been partly due to the diet, but was also influenced by the size of the litter born. Table 1 shows that the average size of the litters decreased from 5.34 in group I to 4.00 in group V. In general the smaller the number of intra-uterine young the more favorable are the conditions for the birth of larger young.

Table 2 gives the average age at weaning and the number and average

weight of each sex in each group when weaned. It is seen that the age at weaning showed a variation of only 4 days, being 24 days in group III and 28 days in group V. The weights of each sex increased as the per cent of protein in the diet was raised. The weights gained by each sex show the same arrangement of augmentation. Since these gains in weight represent different numbers of days the average daily gains in grams and per cents have been computed. These are given in table 2. It is seen that in general the daily gains followed approximately the same order as that of average weights.

The young of group I grew the slowest and often showed marked malnutrition. In many cases the nursing period had to be greatly extended before the young could be weaned. This retardation in the growth of the young was also often associated with blindness and other malformations. The cause of the trouble seemed to be a retarded mammary secretion of the mothers and suggested a lack of vitamin B in the diet. This was discovered early in the experiment. After the addition of a definite amount of yeast to the diet malformations no longer occurred and the young made a better growth. The other groups had occasional malformations until the additional supply of vitamin B had been provided.

The average curves of growth during the first 24 days of the nursing period for the males are shown in figure 1 and for the females in figure 2. It is seen that the males of each group made almost a uniform gain and that the relations of the curves were maintained after the eighth day. The curves of the females were not so uniform and showed greater undulations and crossings of the different groups. Since growth depends on the protein constituency of the diet one would expect that when a copious supply of protein was available the best and most rapid growth would follow. This is indicated by the curves of growth of each sex during the 24 days shown. However, one must not lose sight of at least one other factor which influences the growth of nursing young and which may have been instrumental in modifying the results; that is, the number of young nursed. The young of small litters have a more abundant and available supply of milk than those of large litters. Table 1 shows that the average size of the litters born in the different groups became smaller and smaller from 5.34 in group I to 4 young in group V. These figures, however, do not correctly represent the average number of young which were nursed. Since the mortality of the nursing young occurred usually within a few days after birth, the average size of the litters weaned would more truly represent the number nursed. The average size of the litters weaned in the different groups was: I, 5.60; II, 5.51; III, 4.66; IV, 4.87; V, 4.69. Though the size of the litters nursed doubtless influenced the growth of the young, the differences in the size of the litters were due directly to the per cent of protein in the various diets.



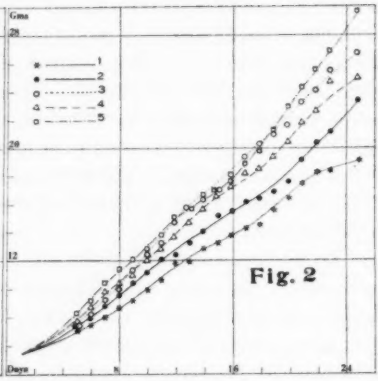
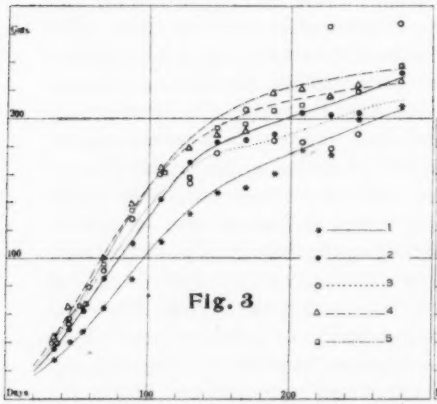


Fig. 1. Curve of growth of males of generation I from birth to 25 days of age. Light solid line and stars, group I; heavy solid line and dots, group II; dotted line and circles, group III; dash line and triangles, group IV; dot-dash line and squares, group V.

Fig. 2. Curve of growth of females of generation I from birth to 25 days of age. Light solid line and stars, group I; heavy solid line and dots, group II; dotted line and circles, group III; dash line and triangles, group IV; dot-dash line and squares, group V.



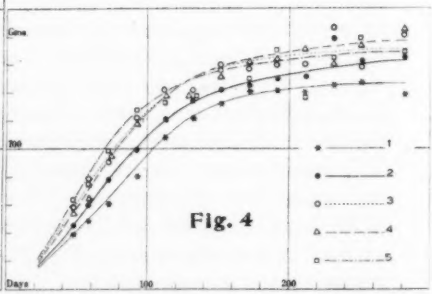


Fig. 3. Curve of growth of males of generation I from 20 to 280 days of age. Light solid line and stars, group I; heavy solid line and dots, group II; dotted line and circles, group III; dash line and triangles, group IV; dot-dash line and squares, group V.

Fig. 4. Curve of growth of females of generation I from 20 to 280 days of age. Light solid line and stars, group I; heavy solid line and dots, group II; dotted line and circles, group III; dash line and triangles, group IV; dot-dash line and squares, group V.

The growth curves of the different groups of generation I from the age of weaning to 280 days are shown in figure 3 (males) and figure 4 (females). Since none of these animals had been mated their weights were not modified by reproductive activities. We have previously shown (Slonaker, 1927) that the activities involved in reproduction modify the weight of the animals thus involved. A direct comparison, therefore, of the growth curves of these virgin animals of generation I with the growth curves (Slonaker, 1931a) of their parents cannot be made. In a later paper we will compare reproducing animals of generation I with their parents.

By comparing figure 1 and figure 3 it is seen that in the males the different groups tended to keep the same relations as exhibited at weaning. A retardation in growth of group III at about 120 days of age caused group II to take third place. The general trend of the curves suggests that had the weighings continued until maximal weight had been reached group II might have occupied first place similar to that exhibited by the parent male. The available cage space made it necessary to discard young before reaching maximal weight to make room for new young.

If the curves of growth of the females shown in figures 2 and 4 are compared it is seen that in all the groups normal growth occurred and that the arrangement at 280 days was the same as at weaning with the exception of groups IV and V which were interchanged at about 140 days of age.

Even though the size of the litter may have influenced the rate of growth of the nursing young, the curves of growth of each sex show that almost the same relationship of weights of the different groups exhibited at weaning was maintained until the age of 280 days. We must therefore conclude that the best growth of young virgin rats was obtained on a diet containing 26 per cent of protein and that in general the rate of growth became less as the per cent of protein in the diet decreased. As stated above the greatest number of young were born in group II and the least number in group V. It is thus seen that the order of the values of the different diets used depends on whether growth, or productivity is considered.

Another interesting observation was noted in the pelage. Both the development of the hair and its condition in the adult was noticeably different when groups I and V were compared. In group I the growth of the hair was slow and scanty as compared to group V. Groups II, III and IV showed intervening conditions. In the adults of group I the hair tended to be coarse and stiff while that of group V was softer and showed a much better condition. This difference might be expected since the hair is rich in nitrogen and its development and condition would depend on the available nitrogen in the diet for its growth.

SUMMARY

Five groups of albino rats (I, II, III, IV and V) fed throughout life on synthetic diets containing 10, 14, 18, 22 and 26 per cent of protein respectively gave the following results in regard to reproduction and the offspring.

1. The order of fertility of both sexes of the parents from highest per cent to the lowest per cent was groups II, I, III, IV and V.

2. The average number of litters and the average number of young born per pair from largest to smallest was in the order of groups II, I, III, IV and V.

3. The sex ratio was highest in group II and lowest in group V. It was below normal in all groups.

4. Results indicate a greater prenatal mortality of males than of females.

5. The mortality of the young during the nursing period was least in group II and greatest in group V. With the exception of group V the mortality of the females was greater than that of the males of the same group. A greater postnatal vitality of the males than of the females is suggested.

6. Cannibalism did not vary sufficiently to warrant any conclusions. It did not exceed the normal.

7. The weight at birth of the males was heavier than that of the females of the same group. In general the weights tended to increase as the protein content of the diet became greater.

8. The per cent of daily gain from birth to the weaning age was lowest in group I and, with one exception, increased to group V with each addition of protein in the diet.

9. The same order of weights found at weaning were, in general, maintained to the age of 280 days.

10. The data show that group II reared a larger number of young which represented a higher per cent of young born than any of the other groups. This was probably due to more vigorous young in group II. The data also show that in general the rate of growth of the young increased as the per cent of protein in the diet increased.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XXIX. Season of Origin as a Determiner of Age at Which Birds Become Sexually Mature

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The factors which influence and collectively determine the age at which birds become sexually mature are very imperfectly known, though it has long been clear that both genetic and environmental factors are involved. More recently the special accelerating action which a hormone of the anterior pituitary bears to sexual maturity in mammals (Smith, and Zondek and Aschheim) was shown to apply also to birds (Riddle and Flemion, 1928); and the writer (1928) obtained some evidence that the thymus and functionally similar structures, prior to their involution, exert a retarding action on sexual maturity. Evans and Simpson (1928) presented evidence that the growth promoting hormone of the pituitary nullifies or restrains the action of the maturity hormone during early life. It should be evident that such hormonal actions are themselves subject to modification by both genetic and environmental influence.

In this paper, and apparently for the first time, it is demonstrated that the age attained by a bird at the time it produces its first egg is much influenced—though probably indirectly—by the *season* in which that bird began life. In view of the enormous amount of work that has been done with the common fowl it is a rather amazing fact that data permitting a similar analysis in that animal seem not to exist.

Material. We here present data for several races belonging to two species of Columbae. These two species are usually classified as belonging to two zoölogical families—Columbidae (common pigeons) and Peristeridae (ring doves). The six races of ring doves (Streptopelia) and the four races of pigeons (Columba) tabulated here are selected solely on the basis of their being strains or races for which we now have most numerous data. One race (T = Tipplers) of the common pigeons used is a long established variety which, like the ring doves, is available for study in many parts of the world. Maturity is reported only for female birds; the test for maturity being the production of the first egg in life, as this was shown either by the external delivery of an egg, or by its falling into the

TABLE 1

The average age (in months) at sexual maturity in females of various races of doves (above) and common pigeons (below) as this is influenced by the season or month of their origin

The integers show the number of birds represented in the average

RACE	DESCRIPTION OF HORIZONTAL LINES	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL
63	Months at maturity	5.8	9.4	8.1	9.7	8.8	8.0	7.3	6.7	6.7	6.0	6.0	5.8	
	Number of birds	5	4	8	6	13	13	14	15	8	7	6	7	109
72	Months at maturity	7.4	9.2	10.2	10.9	9.3	8.2	9.1	7.8	7.2	7.6	6.1	8.8	
	Number of birds	2	8	5	9	11	11	10	8	5	4	2	2	77
N2	Months at maturity	4.6	9.8	12.5	9.2	9.3	9.9	9.1	8.8	6.8	6.9	5.9	6.1	
	Number of birds	3	4	2	8	9	7	6	6	10	2	1	4	61
51	Months at maturity	6.2	14.3	12.5	12.2	12.7	10.3	9.0	9.1	7.5	6.2		9.2	
	Number of birds	2	4	2	9	6	5	10	9	4	2	0	1	54
75	Months at maturity	4.4	5.2	8.9	9.8	8.2	9.2	7.9	7.7	5.7	6.2	10.0	8.3	
	Number of birds	1	2	5	10	7	7	4	2	6	5	2	1	52
62	Months at maturity		8.2	9.1	10.2	9.3	9.2	8.1	7.3	7.4	7.4	5.8	5.4	
	Number of birds	0	4	5	9	11	9	4	3	1	3	1	1	51
Racial monthly mean		5.7	9.3	10.2	10.3	9.6	9.3	8.5	8.0	6.9	6.7	6.8	7.3	
Total birds		13	26	27	41	57	52	48	43	34	23	12	16	404
33	Months at maturity	4.4	9.1	7.7	8.0	7.9	8.3	8.3	7.0	6.3	7.2	5.8	6.0	
	Number of birds	2	3	7	6	10	12	11	9	8	10	6	10	94

PT	Months at maturity	6.4	7.1	7.5	8.3	8.6	8.3	7.1	7.7	6.8	6.6	6.5	5.6	
	Number of birds	4	3	7	5	6	10	13	5	8	7	4	7	79
80	Months at maturity	5.9	7.4	8.8	8.2	8.2	7.1	6.9	7.1	7.1	6.6	6.0	5.7	
	Number of birds	3	2	2	4	9	5	17	9	7	5	4	3	70
T	Months at maturity	5.6	7.8	7.3	5.8	8.1	6.4	7.1	7.2	5.3	6.2	5.4	6.4	
	Number of birds	6	3	5	4	3	7	7	7	7	10	7	7	73
Racial monthly mean		5.6	7.8	7.8	7.6	8.2	7.5	7.3	7.3	6.4	6.6	5.9	5.9	
Total birds		15	11	21	19	28	34	48	30	30	32	21	27	316

body cavity with resulting incubation behavior. Copulation is sometimes necessary to egg production by pigeons, and females not continuously provided with the opportunity to mate with a male are excluded from these data.

All birds considered here were allowed fairly large flying space (5–40 cubic meters) prior to sexual maturity. All of these birds were fed the same dry grain mixture during all months of the year. All young birds were well protected against cold until the age of 55 days; thereafter the ring doves were supplied considerable, the pigeons distinctly less, protection from low winter temperatures. All of the recorded ages are calculated

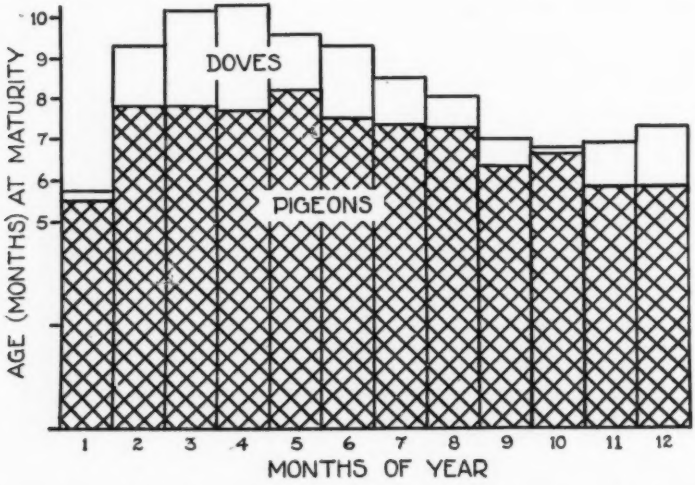

Fig. 1. Polygon showing monthly distribution of average age of maturity in doves and in pigeons. "Months of year" represent months of origin of female birds; the height of the columns indicates the age of these females at the time they produced their first egg.

from the beginning of embryonic development (which, in the case of these birds, coincides with the date on which the egg is laid), not from the time of hatching.

Results. Table 1 supplies the necessary details. Eggs laid in the months of September, October, November, December and January produce birds which, on the average, become sexually mature at a much earlier age than do birds from eggs laid at the other months of the year. Birds hatched from eggs laid in March, April, May and June practically always show, while those from February and July usually show, markedly older average age at maturity. These results hold for both pigeons and

doves, and for the several races of each of these species; any apparent variation from this general rule in any of the 10 races will be found to be based upon an unusually small number of observations. Combining the mean monthly values for November, December and January, and comparing them with similar values for March, April and May, it is found that in the latter months the age of maturity has been increased by 52 per cent in doves and by 36 per cent in pigeons. Such a grouping of 3-month periods of course fails to show the full extent of the seasonal variation.

It is to be noted that the common pigeons mature at a somewhat earlier age than do ring doves—so that a distribution polygon of the latter species is readily superposed upon that of the former (fig. 1). The general result of this study is best shown in this polygon. The tabulated data further show that the average age of maturity is different for some of the races of either species—thus indicating the existence of different genetic factors influencing maturity. For example, smoothed curves constructed from the data for races 63 and 72 would not cross, but remain distinct during all months of the year.

TABLE 2

Seasonal distribution of actual completion of maturity (date of laying first egg) in female doves (above) and pigeons (below)

MONTHS OF THE YEAR												TOTAL
1	2	3	4	5	6	7	8	9	10	11	12	
46	53	74	75	53	26	24	7	15	1	8	22	404
31	43	58	42	37	19	16	6	7	14	14	29	316

Table 2 shows that the birds listed in table 1 actually *completed* their maturity in great numbers at one season (spring) and in very small numbers at another season (autumn). March and April are the center of the period that is evidently favorable for the consummation of maturity; August to December is a period markedly adverse to it. In this connection we note that even mature birds produce less than half as many eggs during October–December as in any other 3-month period of the year (Riddle and Honeywell, 1924). This period is therefore one in which ovarian activity is relatively inhibited, both in mature and immature birds. The fact that the season of numerous completed maturities coincides with a period of decreasing thyroid activity, and the period of fewest completed maturities coincides with a period of increasing thyroid activity, will be discussed immediately.

Discussion. Though, as noted, a hormone of the anterior pituitary is known to exert a major influence on the time of attainment of sexual maturity, I am not aware of any data which either indicate or deny sea-

sonal changes in the production of this hormone. We may therefore think of the pituitary factor as connected, probably primarily, with the genetic differences which establish differences in age at maturity in different races; but, possibly in addition, the output of this hormone may be influenced by season, and the pituitary be thus concerned also in the seasonal fluctuations of age of maturity within any and all races. However this may be, there is definite evidence that seasonal fluctuations in *thyroid* activity are intimately associated with periods of seasonal repression and of growth in the gonads of doves and pigeons—and thus with conditions favorable or unfavorable to the rapid attainment of sexual maturity.

Riddle and Fisher (1925) showed that—in the same colony of doves and pigeons on which the present study is based—the thyroids become definitely heavier (and probably more active) in winter and lighter in summer. In general, largest thyroid size was attained in October, November, December; smallest size was attained in June. Riddle (1925) further demonstrated that the gonads of these same birds undergo seasonal size changes in exactly the opposite direction; testis and ovary enlarge in spring and summer, and become smaller during autumn and winter. In several species of wild birds which breed only in the spring in Florida it has been found (unpublished), however, that the gonads rapidly decrease in size after the breeding season even though the climate is becoming warmer and the thyroids are probably still decreasing in activity; it is therefore fairly evident that, in these species with a very limited breeding period, this decrease in gonad size is not at all associated with an increase in thyroid activity. When the present data for sexual maturity are brought into relation with this earlier work on seasonal size changes in thyroid and gonad in pigeons we note that earliest sexual maturity (from eggs of September–January)—namely, 4 to 6 months—is attained or consummated during February–June. In other words, earliest maturity is actually attained while the thyroid of the maturing bird is *decreasing* in size and activity. The season of origin of a bird effectively modifies its age at sexual maturity; but this is probably accomplished indirectly and through the circumstance that this season of origin determines the length of time that a bird must live before it encounters a season in which endocrine regulation (thyroid, perhaps pituitary) specially favors reproductive activity.

In the vast majority of wild birds sexual maturity (laying of first egg, and first copulations by males) is normally attained in springtime, and therefore probably under conditions similar to those just noted—decreasing size and activity of the thyroid. This is made more probable by the fact that our own observations on reciprocal size changes in the thyroids and gonads of doves and pigeons were later extended to the European crow by Haecker (1926). Unless the domestic fowl is an aberrant form of bird, these same conditions should there similarly and markedly influence the age at which egg-production begins.

SUMMARY

Under standard conditions, in which the temperature factor would seem to be the chief seasonal variable, measurements were made of the age of attainment of sexual maturity (production of first egg) in 404 female doves and 316 common pigeons belonging to 10 different races. Within each of these races some females were reared from eggs laid in each month of the year, and a measure obtained of the influence of this varying season of origin upon age at maturity.

Birds from eggs of September–January, inclusive, matured earliest; those from other months at markedly older ages—36 per cent older in pigeons and 52 per cent older in doves. In general, pigeons mature at an earlier age than do ring doves. Different races within either of these species mature at unequal ages and thus reveal genetic factors, probably involving the anterior pituitary, which also condition the establishment of maturity.

Under the most favorable conditions now known a dove or pigeon must be approximately 4.0 months old before it will produce an egg. If an age of 4 or 5 months is reached during the autumn the maturity is delayed, if reached during February to July maturity is hastened, by endocrine conditions (thyroid, perhaps pituitary) connected with this part of the year. Our earlier studies have shown that this season of accelerated maturity is also the season in which, even in mature birds, thyroid size and activity is decreasing. This rule probably applies to birds generally.

A seasonal factor has been found in practically all aspects of sex and reproduction thus far studied in doves and pigeons.

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SIMULTANEOUS DIRECT AND INDIRECT BLOOD PRESSURE DETERMINATIONS[1]

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It is generally assumed that the direct method of blood pressure determinations yields results comparable to those obtained by the indirect or cuff method. Mummery (1905), Janeway (1909), Warfield (1912), Kolls (1920) and Allen (1923) from experimental work on dogs, and Crile (1903), Merke and Müller (1928) from observations on humans, presented evidence to substantiate this assumption.

The indirect pressure on dogs has been found by Cash (1925–26) to be higher than that obtained by the direct method. Erlanger (1916) noted the appearance of blood from the femoral artery of dogs distal to the constriction at a pressure of 7 to 12 mm. of mercury above where the sounds are first heard.

Dogs under general anesthesia exhibit high direct systolic pressure readings (Dawson, 1906; Cash, 1925–26; and Howell, 1927).

In the following experiments an attempt has been made to correlate the relative value of direct and indirect blood pressure determinations made simultaneously on dogs, both in a normal anesthetized state and after hemorrhage.

EXPERIMENTAL WORK. *Materials and methods.* Twelve dogs, varying in weight from 6.6 to 29.4 kgm., were employed. Five dogs received no preliminary narcosis, one received morphine sulphate 0.015 gram and atropine sulphate 0.0004 gram hypodermatically, while six of the larger dogs received morphine sulphate 0.030 gram and atropine sulphate 0.0008 gram. Uniform ether anesthesia was maintained throughout each experiment, the closed method of administration being employed (Livingstone and Hrdina, 1930).

Direct blood pressure readings were obtained from both the left carotid and left femoral arteries, which were connected by means of three-way glass cannulas with mercury manometers and a kymograph. Indirect determinations were secured by the use of an ordinary sphygmomanometer

[1] This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

band 8 to 10 cm. wide placed around the shaved right hind leg. This air inflated cuff was connected to a mercury manometer.

Procedure: In four animals the cuff was placed on the cannulated leg. Normal indirect and direct readings were obtained simultaneously and recorded on the kymograph. Observations of the beginning flow of blood from the femoral artery during gradual deflation of the cuff were recorded. Note was also made of the beginning pulsation in the femoral artery. Exsanguination was brought about by intermittent bleeding from the carotid or femoral artery. In three experiments 150 mgm. of ephedrine sulphate were administered intravenously when the dog had lost a considerable amount of blood. One animal received an earlier intramuscular injection of 150 mgm. ephedrine sulphate.

RESULTS. Determinations of the indirect blood pressure by this method proved unsatisfactory in four animals, precluding further experimentation. Either no sound could be heard, or it was impossible to get the disappearance of sound regardless of the amount of constriction. In some instances where the indirect determinations were difficult to obtain in unanesthetized animals, they were secured with ease during ether anesthesia.

In 12 experiments the indirect systolic readings in normal anesthetized dogs were from 20 to 100 mm. of mercury higher than the direct. The carotid and femoral direct pressure determinations were quite comparable. Bleeding distal to the cuff occurred at a pressure below but much nearer the indirect than the direct. Thus there was a transmission of sound through the artery after the disappearance of bleeding.

When the blood pressure was lowered by exsanguination, the indirect readings tended to more nearly approximate the direct readings. Following the administration of ephedrine sulphate one animal died, and two presented indirect systolic pressures of 55 and 100 mm. of mercury higher than the direct. The indirect more nearly approximated the direct upon subsequent hemorrhage. Exsanguination was accompanied by an increase in pulse rate in 8 experiments, by a slight increase in 2 animals, and in 2 cases by no increase. A characteristic protocol is as follows:

Dog 127. Male. Weight 23.75 kgm. Morphine sulphate 0.030 gram and atropine sulphate 0.0008 gram were given by hypodermic injection preceding ether anesthesia. The carotid and left femoral arteries were cannulated. A rubber cuff 10 cm. wide was placed around the leg proximal to the point of cannulation of the femoral artery. The cuff was slowly inflated to such a degree as to cause the disappearance of the pulsation of the femoral artery tracings as recorded on the kymograph. The side outlet on the femoral artery cannula was now slowly opened and the femoral kymographic tracing fell to the base line. The cuff was then slowly deflated and the pressure at which the first clear sounds were heard was recorded as the indirect systolic pressure, or 240 mm. of mercury (fig. 1). The direct carotid pressure at this time was 142 mm. of mercury, while the direct femoral pressure was 140 mm. of mercury. Slow deflation of the cuff was continued until blood appeared from the peripheral cannula,

at which point the pressure reading was noted (224 mm. of mercury), and the cannula outlet closed. After the removal of 700 cc. of blood the indirect systolic pressure was 108 mm. of mercury and the direct carotid pressure was 68 mm. of mercury. Bleeding began at 92 mm. of mercury. One minute later 150 mgm. ephedrine sulphate were administered subcutaneously. Practically no change in the tracing occurred, so 6 minutes later another 150 mgm. ephedrine sulphate were administered intrave-

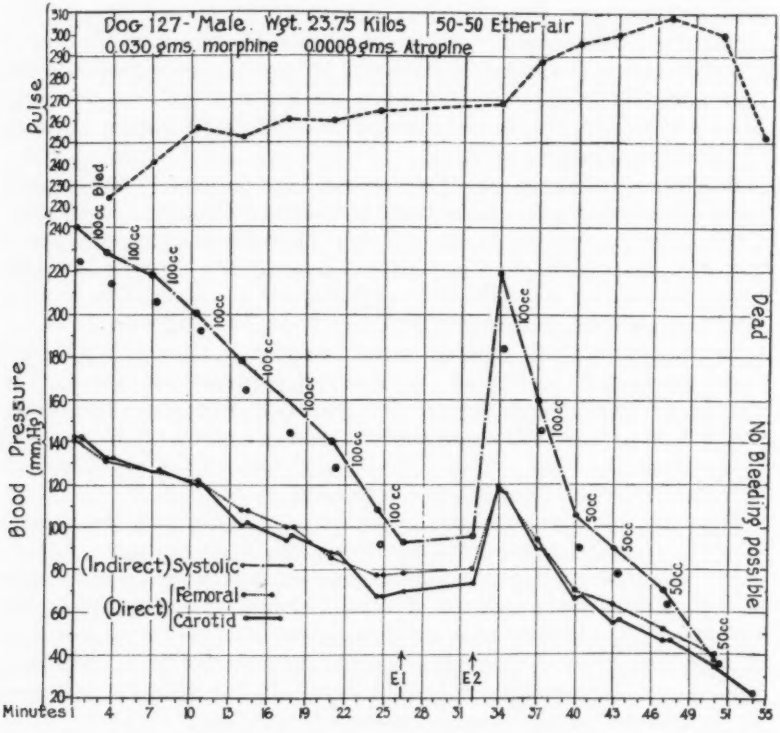

Fig. 1. Direct and indirect blood pressure and pulse curve during exsanguination. ⊙, indicates points where bleeding began; E_1, intramuscular injection of 150 mgm. of ephedrine, and E_2, intravenous injection of 150 mgm. ephedrine.

A preliminary report of this study was published in Proc. Soc. Exper. Biol. and Med., 1930, xxviii, 130.

nously. Two minutes later the readings were as follows: indirect systolic pressure of 220 mm. of mercury, and direct carotid pressure of 118 mm. of mercury. Bleeding began at 184 mm. of mercury. The pulse was 268.

Bleeding was continued at intervals, being accompanied by a repetition of the above procedure. The dog expired after the removal of a total of 1100 cc. of blood.

COMMENT. The popular type of glass cannula was employed rather than a maximum and minimum pressure valve (Dawson, 1906; Kolls, 1920; Howell, 1927; and McGregor, 1928), as we were not attempting to determine the absolute systolic and diastolic direct pressures. Our interest was in determining the relative values in terms of millimeter of mercury of the pressure readings obtained by means of the sphygmomanometer and the mercury manometer.

The present study is based on a series of only 12 dogs, but as several rather startling facts have appeared, they are presented at this time, without any attempt at generalization.

It is of interest to note the enormous difference between the direct and indirect blood pressure readings obtained simultaneously in a normal anesthetized dog, and the slight difference between these two determinations when the animal presents a low blood pressure. This tends to suggest that the determinations made on an animal with a low blood pressure are perhaps more accurate. These findings cause one to be skeptical of the height of the blood pressure in clinical cases of hypertension.

CONCLUSIONS

1. Indirect blood pressure readings in anesthetized dogs were found to be 20 to 100 mm. of mercury higher than the direct.

2. Bleeding occurred at a pressure below but much nearer the indirect than the direct.

3. When the blood pressures were markedly lowered by exsanguination from the carotid artery, the indirect pressure readings tended to more nearly approximate the direct readings.

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THE EFFECTS OF PROGRESSIVE SYMPATHECTOMY ON BLOOD PRESSURE

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The close dependence of vascular tone on sympathetic impulses as shown by more or less persistent vasodilatation when the sympathetic nerves to arterioles are cut, the importance of vascular tone for the proper distribution of blood in the body, and the recent increase in the application of surgery to the sympathetic system render important an enquiry into the effect of sympathectomy on blood pressure. The work of W. B. Cannon, Lewis and Britton (1927) proved that animals can survive total abolition of sympathetic activity in the body. It was clear, therefore, that a study could be made of the effects on blood pressure of extirpating not only parts of the sympathetic system but the whole system as a distributor of tonic impulses to blood vessels.

METHOD. For the present study dogs between 11 kgm. and 13 kgm. in weight were used. They were found large enough to allow accurate readings of the blood pressure and small enough to make operating possible. They were trained to lie quietly on a table during the blood-pressure determinations. A typical rubber cuff, reduced in width to about 8 cm., was used, and the pressure was measured by means of a mercury manometer. The cuff was applied to the leg below the knee; a small diaphragm stethoscope, or palpation, or both, were used to determine the disappearance and reappearance of the pulse. The artery (dorsalis pedis) on the anterior surface of the foot served for the observations. By this method it was possible to get reasonably consistent results in repeated observations within a brief period under similar conditions. Care was taken to place the cuff as nearly as possible in the same position in successive readings. The heart rate was recorded with the blood pressure.

With one exception the parts of the sympathetic system were removed in the same order. The first step was the removal of both abdominal chains from the diaphragm to the sacrum and the severance of the right splanchnics at the diaphragm. The second was the removal of the right thoracic chain from the stellate ganglion to the diaphragm through which were pulled the remnants of the splanchnics. The final operation involved the removal of the left thoracic chain from the stellate ganglion to the dia-

phragm where the left splanchnics were cut. The animals recovered rapidly from the operations, and in each instance the readings were resumed on the following day.

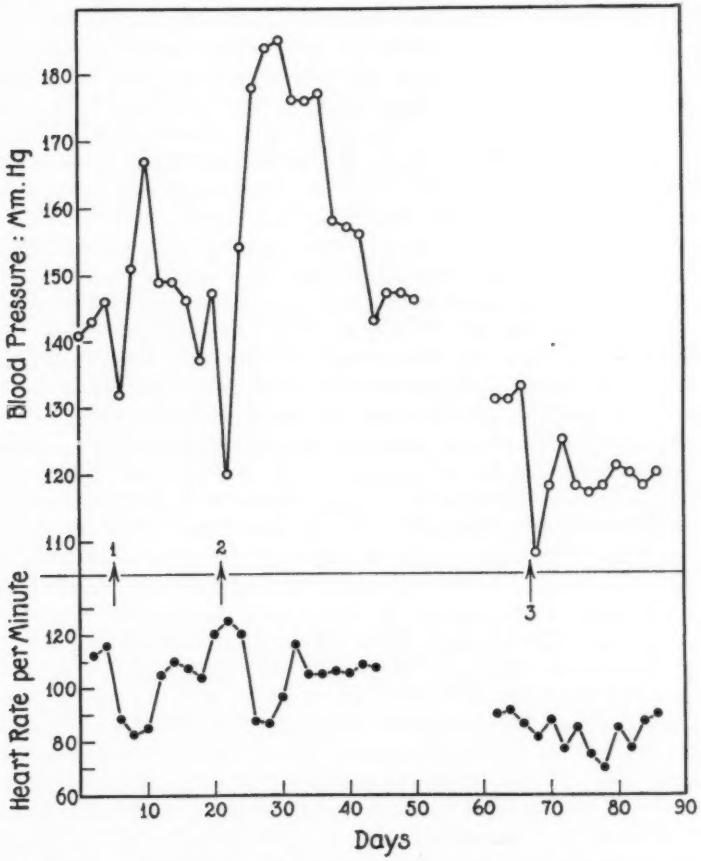

Fig. 1. The graph has been condensed by averaging the readings of two successive days (1 and 2, 3 and 4, etc.), so that there is a two-day interval between recorded readings. In each instance, however, the first record after operation is that for the next day. The numbers refer to the three steps of the sympathectomy described in the text.

RESULTS. The most complete case is recorded graphically in figure 1. The pre-operative blood pressure varied between 140 and 145 mm. Hg and the heart rate between 106 and 118 beats per minute. After extirpating

the two lumbar chains and cutting the right splanchnics the blood pressure fell to 132 mm. Hg (the next day) and gradually rose until it reached 167 mm. Hg on the sixth post-operative day. It then slowly fell to about its previous average level, near 145 mm. Hg. After removing the right thoracic chain, the blood pressure dropped to 120 mm. Hg on the first post-operative day; it then rose rapidly to 178 mm. six days later and more slowly to a maximum of 185 mm. four days after that. The rise was followed by a gradual fall to a pressure about 10 mm. lower than the previous average. The final operation, excluding from action the left thoracic chain and left splanchnics, caused a drop to 108 mm. Hg on the first post-operative day, followed by a rise to 125 mm. on the sixth day and a prompt fall to an average of about 120 mm. which continued.

The rise of blood pressure after the first and second operations (fig. 1) was associated with a reduction of the heart rate—a result in accord with Marey's law and with recent evidence of reflex bradycardia from stimulation of the nerve endings in the carotid sinus (Heymans, 1929). The marked fall of pressure after the second operation was associated with a rise of the rate, but a deeper fall after the third operation was not thus associated. In both instances the vagi were intact; but in the latter instance the sympathetic supply to the heart had just been destroyed by removal of the final remnant of the sympathetic. The evidence that hypotension induces medulli-adrenal secretion (see Heymans, 1929, p. 54) shows that the sympathico-adrenal system is brought into action when the arterial pressure falls. The difference in the effects of the pressure-drop on the cardiac rate, attending a difference in the sympathetic supply to the heart, raises a question as to whether in hypotension the sympathetics are not more important than the vagal influences in causing acceleration.

The observations illustrated in figure 1 were confirmed in three other dogs in which the results were essentially similar. For example, in a white Spitz, the slow rise of pressure after abdominal sympathectomy amounted to about 15 mm. Hg; after right thoracic sympathectomy the pressure rose gradually about 25 mm. above the pre-operative level and then gradually returned; and after the final operation, with cutting of the left splanchnics, it fell about 30 mm. (to 108 mm. Hg) and rose thereafter to a permanent level at about 130 mm. Hg. In another instance, the order of the operations was right thoracic sympathectomy with right splanchnectomy, then lumbar sympathectomy with left splanchnectomy. It was significant that the blood pressure rose after the second operation to a range about 30 mm. above the pre-operative normal level, although all sympathetic control of splanchnic vessels and of adrenal secretion had been abolished.

The completely sympathectomized animals relied on shivering when they were exposed to a cool atmosphere, evidently a consequence of failure of the vasomotor function. No coincident effect on blood pressure was observed.

The interesting and rather surprising feature of the foregoing results is the rise of blood pressure to supernormal levels when a large part of the sympathetic control of blood vessels is excluded. It might be supposed that possibly the outlying neurones of the splanchnic area, when released from control by section of preganglionic fibres, became overactive and thus caused the higher pressure. But the same phenomenon appeared after removal of the right thoracic sympathetic when only the outlying neurones of the cervical distribution were isolated, and it did not occur after removal of the left thoracic chain when the splanchnic and the cervical regions were both isolated. It seems probable that the phenomenon is due to an overcompensation by the remnant of the sympathetic system, at each step except the last, for the part that has gone. How the effect is produced is not clear.

Rowntree and Adson (1929) have reported a case of Raynaud's disease of the hands in which a bilateral cervico-thoracic ganglionectomy was performed. A few years previously the patient had had a bilateral lumbar ganglionectomy for the same condition in the legs. Thus the patient was without vasomotor control in the four extremities and the head. A series of blood pressure readings was recorded after the cervico-thoracic ganglionectomy; three weeks after the operation the blood pressure, which had been 120/80 mm. Hg, had risen to 175/110; it returned gradually to the previous normal average (about 120/80) recorded four months after the operation. Their observation has a resemblance to those recorded above on dogs.

SUMMARY

After removal of the lumbar sympathetic chains and severance of the right splanchnics, and again after removal of the right thoracic sympathetic chain from dogs, the blood pressure undergoes an initial fall which is followed promptly by a rise which gradually reaches a level considerably above normal before it slowly returns to approximately the previous normal range. After complete exclusion of sympathetic control of the blood vessels (by final extirpation of the left thoracic sympathetic chain and severance of the left splanchnics) the blood pressure drops lower than after the previous operations and then returns to about the normal level, without any noteworthy rise above that level (see fig. 1).

Extreme changes of blood pressure, before removal of the last of the sympathetic connections, were accompanied by inverse changes of the heart rate (see fig. 1).

I wish to express appreciation to Dr. W. B. Cannon for suggesting this study and for help in the operations.

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AN EXPLANATION OF THE RESULTS OF THE "ALCOHOL BLOCK"

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The "alcohol block" method has been extensively used in investigations on nerve impulses. The assumption was originally made that an impulse would suffer progressive decrement in traversing a narcotized portion of the nerve, and that a subnormal impulse would be extinguished earlier than a full-sized one. Since subnormal impulses occur when the rate at which impulses pass along a nerve is so fast that each falls into the relative refractory phase of its predecessor, it was thought that the alcohol block method would give information as to reflex frequency (Forbes and Olmsted, 1925). In the decerebrate cat, if an alcohol block is applied to the popliteal nerve and responses taken from *soleus* (Liddell and Olmsted, 1929) or to the femoral nerve and responses taken from *quadriceps* (Olmsted and Ball, 1929), the disappearance of responses is in the following order: 1, stretch reflex; 2, jerk; 3, crossed extension reflex; 4, response to direct stimulation of motor nerve. On the above assumptions, it was thought that this order of extinction of responses would indicate the relative rates at which impulses were passing over the motor nerve in these reflexes. Kato (1924), however, has shown that except over a very short distance, some 3 mm. each side of the boundary of the narcotizing chamber, neither full-sized nor subnormal impulses suffer progressive decrement in traversing a narcotized length of nerve, and both full-sized and subnormal impulses are extinguished at the same time. There was, therefore, a serious fallacy in the argument underlying the explanation of the action of the alcohol block and doubt has now arisen as to the reliability of information regarding reflex frequency obtained by application of this method. "The failure of the reflexes under the alcohol block must be due to some other cause than the high frequency of discharge" (Adrian and Bronk, 1930).

Two other hypotheses deserve consideration: the order of falling out of responses under the alcohol block may be due 1, to the rate of penetration of the alcohol into the nerve, or 2, to differences in susceptibility of individual fibers to the narcotizing agent.

If we assume, on the first hypothesis, that the alcohol penetrates the

nerve at a fairly uniform rate and that all the fibers are equally susceptible, then when the alcohol block is applied the outermost ring of fibers should become narcotized first, a narrower ring containing fewer fibers would then succumb, and so on until finally the few remaining central fibers would cease to function. If the block were applied to a motor nerve, the falling off in tension in response to maximal motor stimulation should be rapid at first, but become slower and slower until eventually the muscle ceased to respond. On the second hypothesis, we may suppose that the alcohol comes into contact with all the nerve fibers practically simultaneously. If different fibers differ in their susceptibility to alcohol, one would expect that the smaller fibers would be less resistant than the larger. Eccles and Sherrington (1930) assume that the contraction-tension value for a given nerve fiber runs parallel with its size. Hence on the second hypothesis, falling off in tension after application of the alcohol block should proceed slowly at first, then speed up and reach its most rapid rate just before complete extinction. It should be possible, therefore, by following the rate of falling off in tension during the application of an alcohol block to determine if either of these two hypotheses can explain the results.

Eccles and Sherrington (1930) have found that the diameters of the different fibers composing typical motor nerves to the hind limb of the cat range in size from 2–20μ. When the diameters are plotted against numerical distribution it is found that they fall in each case into two distinct groups, one having its peak towards the lower end of the scale, the other towards the upper; thus for the nerve to *gastrocnemius medialis* the peaks are at 5–6μ and 14–15μ; for *gastrocnemius lateralis*, 4–5μ and 14–15μ; for *extensor longus digitorum*, 4–5μ and 12–13μ; and for *soleus*, 4–5μ and 10–11μ. These authors suggest that the number of muscle fibers innervated by a given motor nerve unit depends directly on its cross-sectional area, and therefore that the contraction-tension value for a given motor unit will vary directly with the cross-sectional area of its nerve fiber. They have calculated (table V, column 6) the cross sectional area of each group of fibers in the nerve to *gastrocnemius medialis* as percentage of total area. If, upon application of an alcohol block to the nerve to *gastrocnemius medialis*, the groups of fibers become narcotized in order of increasing size, the falling off in tension of the muscle in response to a maximal motor nerve stimulation should not follow a smooth curve but should follow a curve like that shown in figure 1 (broken line). Since all the fibers up to 12μ would be responsible for only 10 per cent of the total tension, the falling out of all the smaller fibers would cause very little diminution in tension; because of the two peaks in the size-distribution curve three distinct phases in falling off in tension should be found: 1, early in narcosis a very slight drop; 2, a period when the tension should change but little; 3, a very rapid fall leading to extinction. From the

data of Eccles and Sherrington (1930) we have calculated the contraction-tension values for motor units of the nerves to *gastrocnemius lateralis*, *extensor longus digitorum*, and *soleus*. The curves showing the theoretical falling off in tension, as the fibers of these three nerves cease to function in order of increasing size, are given (together with that for *gastrocnemius medialis*) in figure 1. The same general features are seen in each curve; in addition it is to be noted that total extinction should occur earlier in *soleus* than in the other three muscles. Figures 1–4.

We have used the decapitate cat in which to follow the falling off in tension of these four muscles, and of *tibialis anticus* and *plantaris* as well.

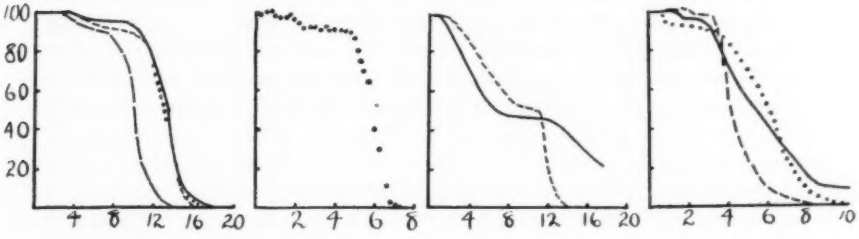

Fig. 1 Fig. 2 Fig. 3 Fig. 4

Fig. 1. Ordinates = per cent of total cross-section area of nerve fibers remaining after subtraction of cross-section area of individual groups in order of increasing size. Abscissae = fiber diameter in μ. Plotted from the data of Eccles and Sherrington. *Gast. lat.* ——, *gast. med.* ----------, *ext. l. dig.* . . . . *soleus* —— ——.

Fig. 2. Ordinates = per cent tension of soleus in response to maximal motor nerve stimulus during application of 15 per cent alcohol block. Abscissae = time in minutes after application of block.

Fig. 3. Ordinates = per cent tension in response to maximal stimulus to *popliteal n.* during application of 15 per cent alcohol block. Abscissae = time in minutes after application of block. *Gast. med.* ——, *soleus* —— ——.

Fig. 4. Ordinates = per cent tension in response to maximal motor stimulus to undivided sciatic nerve during application of 15 per cent alcohol block. Abscissae = time in minutes after application of block. *Gast. med.* and *lat.* (together) ——, *plantaris* . . . ., *soleus* —— ——.

The alcohol was applied in the form of a cotton swab 0.5 cm. wide and 2 cm. long, soaked in warm Ringer solution containing 13 to 15 per cent alcohol. The whole sciatic or its divisions, popliteal and peroneal, separately, were severed as high in the thigh as possible and the peripheral end enclosed in shielded electrodes sewed to the muscles to hold them in place. The block was applied between the electrodes and the knee about 1 cm. below the glass shield. A drill through the femur near the knee, and another through the ankle held the limb rigid. The tendons of the muscles in question were cut, the foot amputated, and the muscles then dissected as free from each other as possible without damaging blood supply, etc.

The tendons were attached to levers which wrote on a smoked drum, and records were made of the contraction heights. The levers used were not strictly isometric, since the spring against which they pulled allowed a movement of slightly less than 2 mm. for each 100 grams of tension. The levers were calibrated so that the tension could be expressed in grams. In some of the earlier experiments single maximal break shocks were used, but in the majority of experiments a tetanizing current from a Harvard inductorium was used, the current being maintained just long enough to be certain that a maximal response had been obtained. The nerves were stimulated at 15 second intervals.

It was found that 15 per cent alcohol was a favorable length for our purposes, since it was practically always sufficient to cause the tension in *soleus* to disappear completely, though this was not true for the other muscles. A curve showing the falling off in tension of soleus when a block of 15 per cent alcohol was applied to the popliteal nerve is shown in figure 2. The resemblance to the theoretical curve (fig. 1, broken line) is marked. A total of forty-five curves was plotted. In some the initial break occurred very early in the curve; in some the nearly level plateau was replaced by a gradual slope; in some the plateau occurred lower down (for examples of these features see fig. 3); but the same essential characteristics were noted in all. In no case did any curve follow the course it would have done had the falling off in tension been due to the falling out first of a large number of fibers, then of fewer and fewer fibers.

It was also found that when the actions of two, three, or four muscles were recorded simultaneously, e.g., *gastrocnemius medialis* and *soleus* (fig. 3), undivided *gastrocnemius, soleus, extensor longus digitorum* (fig. 4), *soleus* invariably ceased to contract first. *Extensor longus digitorum, tibialis anticus,* and *plantaris* always ceased to contract somewhat later than *soleus,* but as a rule earlier than *gastrocnemius.*

We followed the return of contraction after removal of the alcohol in fourteen cases. Recovery to about 50 per cent of the maximal tension was very rapid in every case; after this the rate was slowed. These curves are never the exact mirror images of the curves showing disappearance of tension, but they show that the tension returns in essentially the same manner that it disappears.

We have invariably found, however, that when records are taken from *soleus* simultaneously with other muscles, the first small break in the curve for *soleus* occurs later than in the curves for the other muscles (figs. 3, 4) and to a less degree than one would expect from the theoretical curves. The difference in time may be due to some peculiarity in rate of penetration of the small fibers of this nerve or these fibers may be larger than expected from the counts of Eccles and Sherrington; the difference in degree may indicate that these small fibers do not control so large a propor-

tion of the total contraction-tension as expected. It must also be borne in mind that soleus is a "red" muscle, the others are pale or mixed.

In spite of this discrepancy between the theoretical and experimental curves for *soleus*, the general shape of all the curves and the fact that *soleus* invariably ceases to contract earlier than the other muscles show that the experimental results meet very well the demands of our second hypothesis, viz., that the different nerve fibers become narcotized in order of increasing size. If this is true for motor nerve stimulation, it should hold for reflex stimulation as well. This would indicate that the stretch reflex and je[illegible]nvolve only the smaller fibers in the motor nerves, since, as shown in [illegible]gure 3 of the paper by Liddell and Olmsted (1929), the former disappears when there is very little loss in total tension, and the latter is reduced nearly to zero when the muscle is still capable of exerting three-fourths its total tension. This would imply that only the smaller motor nerve fibers would be involved in maintenance of postural tone.

CONCLUSION

Evidence is given to support the conclusion that the effects of "alcohol block" are due to the progressive narcotization of individual nerve fibers in order of increasing size.

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URINE FLOW AND DIURESIS IN MARINE TELEOSTS[1]

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There is reason to believe that most of the rates of urine flow obtained on marine teleosts under experimental conditions are much higher than under normal conditions of existence (Marshall, 1930; Smith, 1930). Flows reported in the literature (calculated per kilogram per day) are as follows: wrymouth (8 specimens)—1.5 to 11.0 cc., sculpin (16 specimens)—3.2 to 57.0 cc., goosefish (11 specimens)—12.9 to 54.4 cc., toadfish (15 specimens)—0.6 to 9.4 cc., (Marshall, 1930); goosefish—20 to 30 cc., Scorpoena—10 to 12 cc., Conger—3 to 5 cc. (Burian, 1909); goosefish—18 cc., Muraena—5 cc. (Edwards and Condorelli, 1928); sculpin—11 to 40 cc., Anguilla—0.4 to 5.0 cc. (Smith, 1930). The recent hypothesis of Smith (1930) that the aglomerular condition of marine teleosts is related to the peculiar water cycle associated with a marine habitat demands a very low urine flow in marine teleosts as compared to fresh water species. The present investigation was undertaken to determine: 1, the rate of urine flow of marine teleosts under normal conditions; 2, the factors responsible for causing the abnormally high urine flows frequently observed; and 3, the nature of the diuresis occurring under these conditions.

The toadfish (*Opsanus tau*) and the sculpin (*Myoxocephalus octodecimspinosus*) have been used exclusively. The toadfish has an aglomerular kidney (Marshall, 1929), the sculpin a glomerular kidney. The toadfish kidney has recently been shown to possess but one type of renal epithelium provided with brush border (Grafflin, 1931). The work on the toadfish was done at Baltimore, that on the sculpin at Salisbury Cove, Maine. Most of the work on the toadfish was done early in the investigation when the factors entering into the problem were not fully appreciated, so that the results here reported are based for the larger part upon work on the sculpin. The sculpins were always in sea water with a chloride concentration of approximately 500 millimols per liter. Toadfish were kept in brackish river water with a chloride concentration varying from 100 to a little over 200 millimols per liter. Urine was obtained by inserting

[1] I am indebted to a "Special Fund for Physiological Research" for part of the expense of this investigation.

a fine glass catheter into the urinary papilla and expressing the urine by massaging the lower abdomen. When urine flow was to be determined the bladder was first emptied and the urinary papilla then tied with silk thread. At the end of the period, the thread could be cut without injury to the papilla and thus many collections could be made on the same fish. All flows reported in this paper are calculated on the basis of cubic centimeter per kilogram per 24 hours. Sculpins were bled from the ventral aorta, which is very easily exposed in this species. Heparin was used exclusively as an anticoagulant. Total nitrogen in the urine was determined by the method of Van Slyke (1927); urinary and plasma chloride by the method of Van Slyke (1923); and urinary sulphate by the benzidine method. Chloride and sulphate are reported as millimols per liter. Total nitrogen is reported as milligrams per cent. Duplicate determinations were always run where sufficient fluid was available.

That experimental manipulations have a pronounced effect on the rate of urine flow can be shown by the fact that where the flow was followed over a period of days marked variations occurred. In these

TABLE 1

DATE	FLOW	URINARY Cl	URINARY SO_4
December 4—urine in bladder		86	119
December 4–5	3.6	102	94
December 5–6	5.1	117	85
December 6–7	11.2	165	48

experiments the fish were wrapped in a towel on being caught, were held thus out of water until the manipulations had been carried out, and were kept in a large wooden live car or a well-aerated aquarium between times.

Table 1 gives the data on a toadfish handled as described. The urine was collected in periods of approximately 24 hours each. In no case was there any possibility of overdistention of the bladder. The water used had an average chloride concentration of 170 millimols and an average sulphate concentration of 11 millimols per liter. The temperature of the water averaged 20°C.

Tables 2 and 3 give the data obtained under similar conditions for two sculpins. The tying periods varied from 2 to 6 hours, and the temperature of the water averaged 15°C.

This type of experiment, many times repeated, shows clearly that the urine flow steadily increases when followed for some days, with the suggestion in table 2 that the flow may diminish again after reaching a peak. With the diuresis the concentration of urinary chloride increases tremen-

dously, the total nitrogen percentage shows a marked decrease (in one sculpin from 222 to 13), and the total SO_4 always increases, although the percentage SO_4 may decrease, increase, or remain almost unchanged. The data on SO_4 for the toadfish clearly indicate a steadily decreasing SO_4 concentration as typical, but the opposite has been found on several occasions. The data on SO_4 for sculpin are incomplete, particularly in the early stages of the diuresis, but point to either a steadily decreasing or an almost unchanging SO_4 concentration. In no instance in the sculpin has the SO_4 been observed to increase in concentration as the experiment progressed. Analyses for phosphate in the urine, though incomplete,

TABLE 2

DATE	FLOW	ANALYSES OF URINE		
		Cl	Total N	SO_4
July 16—original urine	10.4		107 82	
July 20	51.9	231	28	32
July 22	71.0	259	11	29
July 23	52.6	301		

Plasma chloride after last period—212. Weight 162 grams.

TABLE 3

DATE	FLOW	ANALYSES OF URINE		
		Cl	Total N	SO_4
July 16	11.9	26		
July 20	71.2	224	8	31
July 22	112.0	239	7	21
July 23		310		

Plasma Cl after last collection—225. Weight 155 grams.

especially in the early stages, have made it quite clear that in both the toadfish and the sculpin the urinary phosphate concentration unquestionably diminishes as the diuresis progresses. It must be borne in mind that we are dealing with two entirely different types of kidney, one glomerular (sculpin) and one aglomerular (toadfish). Also, it must be remembered that the sculpin is in sea water with a SO_4 concentration of 30 millimols per liter, the toadfish in river water with a SO_4 concentration of about 11 millimols per liter.

Experiments of this type suggested that the high urine flows encountered under experimental conditions are the result of damage to the fish, most

likely caused by handling. To test this possibility, a series of analyses was made of plasma and urine obtained from sculpins as soon as they were removed from their natural habitat. Since only small amounts of urine could be obtained from the bladder, only determinations of the chloride and total nitrogen of the urine were carried out. These results showed without exception: chloride, zero to trace; total nitrogen, over 100 mgm. per cent (as high as 287 mgm. per cent). These findings were in distinct contrast to the very high chloride and very low total nitrogen frequently encountered in the urine obtained from fish under ordinary experimental conditions. Also they were in keeping with the observations of Grollman (1929) that the urine taken from goosefish off St. George's Banks in the Atlantic was high in total nitrogen and low in chloride. In the plasma, chloride alone was determined, and in a series of six sculpins bled immediately after being caught, the chloride content of the plasma was found to be absolutely constant at 151 millimols per liter (150 to 153). This again is in striking contrast to plasma chlorides of 200 and over frequently encountered in fish which had been subjected to experimental manipulation (see tables 2 and 3).

We, therefore, decided to take as criteria of normality of sculpins 1, a plasma chloride around 150 millimols per liter; 2, a urinary chloride of zero or trace, and 3, a urinary total nitrogen of 100 mgm. per cent or over. As will be shown later, it is probable that a fish may satisfy all of these criteria and still show a slightly elevated urine flow.

Handling, with its possible associated skin injury, immediately suggests itself as being connected with the abnormalities encountered previously in experimental fish. To test this idea, two types of experiments were undertaken. In the first type, a batch of sculpins were subjected to skin injury by scrubbing vigorously with a hand brush as soon as they were caught. These fish were never out of water, to avoid asphyxia as a complicating factor. Daddy sculpins (*M. scorpius*) scrubbed for three minutes were found to have plasma and urine chlorides over 200 millimols per liter within 24 hours after such treatment, as contrasted with low plasma and urine chlorides in controls. Daddy sculpins scrubbed 1 to 1½ minutes gave similar results at the end of 48 hours. The same results were obtained on ordinary sculpins (*M. octodecimspinosus*), with plasma and urine chlorides over 300 millimols per liter in one instance. On the other hand, sculpins kept in an aquarium with mud bottom for 8 days showed urinary chloride below 100 and plasma chloride below 170 millimols per liter. Increasing pigmentation and dulling of the skin are very striking in sculpins 24 hours after scrubbing.

A second type of experiment to test this idea was to attempt to keep a batch of sculpins under conditions such that extreme precautions were taken to avoid skin injury. To this end, one of the deep wooden 'ive

cars used previously for the sculpin work was lined with white oil cloth, multiply perforated with a cork borer to allow free circulation of water, and swung so that the cloth touched the car only where it was tacked around the sides. Needless to say, the smooth surface of the oil cloth was exposed to the fish. Sculpins were handled as follows: as soon as they were caught (on small hooks) they were brought to the surface and dipped up in a bucket, never being out of water. The hook was removed under water by propping the mouth open with two fingers held inside the mouth and gently working the hook loose to avoid tearing the flesh. If there was bleeding at the point of hooking, the fish was discarded. The sculpin was then immediately transferred to the oil cloth car, where he was left unmolested. In this way, the skin was touched only by the surface of the bucket and by the smooth surface of the oil cloth. Two sculpins handled in this manner and kept in the oil cloth car for eight days showed plasma chlorides of 150 and 151. Two sculpins handled in the same way but kept in a car not lined with oil cloth showed plasma chlorides of 152 and 155.

TABLE 4

NUMBER	WEIGHT	FLOW	PLASMA Cl	URINARY Cl	URINARY TOTAL N
4	179	1.0	165	0	
2	196	3.4	160	0	80
6	203	3.8	165	40	112
1	176	3.8	162	0	129
3	150	10.7	157	0	206
5	161	15.3	166	36	251

This was satisfactory evidence that, if extreme precautions were taken to avoid skin injury, the plasma chloride of sculpins kept in captivity for a week or more was essentially normal. An attempt was now made to measure the urine flow under similar precautions to avoid skin injury. The fish were put in a shallow porcelain bowl for easier handling during manipulations of tying and collecting urine. They were grasped as gently as possible by the thumb and index finger on the tail just posterior to the anal opening and rolled over in the dish, being grabbed by two fingers of the other hand under the gill coverings. The region of the urinary papilla was held above the level of the water for tying and collection, but the gills were always kept under water. Collection periods of 24 hours were used. The results on 6 sculpins so handled are given in table 4. In only one instance, sculpin 5, is there any possibility of overdistention of the bladder. It is unfortunate that the blood samples for chlorides could not be obtained until 2 to 3 hours after the urine collection.

In 4 of the 6, the flow was below 4 cc. per kgm. per 24 hours. Since it is obvious that in determining these flows even under the best conditions it is unavoidable that some injury to the fish must result, these may possibly be maximum flows. We find the urinary chloride zero or very low, and the total nitrogen apparently increasing with increasing rate of flow. The plasma chloride is distinctly higher than in the normal series reported above. Results on other fish make it almost certain that these results

TABLE 5

WEIGHT	FLOW	URINARY Cl	URINARY SO_4	URINARY TOTAL N
360	0.95	0		133
410	1.5	0	65	150
350	2.2	0		190
565	2.2	0		64
395	3.8	58	99	71
475	5.0	13	94	67

TABLE 6

NUMBER	WEIGHT	FLOW	PLASMA Cl	URINARY Cl	URINARY TOTAL N
			Oil-cloth car		
5	175	11.5	163	0	237
6	180	9.0	150	0	222
7	177	7.0	157	0	286
8	230	17.2	157	125	136
9	187	4.1		23	150
			Non-oil-cloth car		
6	184	6.5	153	80	222
7	193	2.6	160	72	315
8	172	3.6	161	11	361
9	200	4.8	152	44	182
10	188	3.9	154	25	153

would be distinctly lower if the blood samples had been taken right at the end of the collection period.

It is interesting to compare with this table a series of results obtained by the towel technique on toadfish as soon as they were brought in from the traps. The tying periods were approximately 17 hours in length. These results were obtained in the month of June, and are given in table 5.

Another experiment was carried out in which 9 sculpins, handled with extreme precautions as described above, were put into the oil cloth car and

10 sculpins, similarly handled, were put into a car not lined with oil cloth and scrubbed free of algae just before the experiment was started. These fish were left untouched for 18 days. On the 18th day 4 sculpins from the oil cloth car and 5 from the ordinary car were bled at once. None of the plasma chlorides was much above normal and the average for the oil cloth car was somewhat lower than that for the non-oil-cloth car. The highest plasma chloride in the non-oil-cloth car was 159 millimols per liter. On the remaining fish, urine flows were determined the fish being handled by the technique described above. Blood samples were taken without delay at the end of the collection period. The sculpins were tied off for 24 hours. The results of the experiment are given in table 6. In only one case (no. 8, oil cloth) is there a possibility of overdistention of the bladder. The results obtained on fish after eighteen days of captivity are essentially the same as those obtained on fish caught just before being studied.[2]

An attempt was now made to follow the urine flow for some time under the best possible conditions of handling to avoid injury to the skin, according to the procedure outlined above. These experiments demonstrated conclusively that the urine flow will almost invariably increase in spite of extreme precautions. The results on one sculpin tied up for two successive periods of about 2.5 hours each immediately after catching were as follows: 1st period, 1.5 cc.; second period, 6.2 cc.

We have tabulated all of our urine flows on the sculpin, along with the urinary chloride and total nitrogen. These flows are 76 in number and vary from 1 cc. to 112 cc. per kilogram per 24 hours. When one comes to analyze the data, one finds flows recorded early in the experimental life of a sculpin (first 24 hours) varying from 1 to 28.9 cc., with urinary chloride always below 100 except in one case (128). All of these flows except 3 show a urinary total nitrogen well over 100 (up to 360 mgm. per cent). On close scrutiny, one finds that the flows between 1 cc. and 28.9 cc. which show high chloride (up to 340) and low total nitrogen were all determined 1, late in the experimental life of the fish, when the diuresis was on the decrease; 2, after the skin of the fish had been seriously injured by scrubbing, or 3, after a stay of some length in the live cars. All of the flows above 28.9 cc. show a high urinary chloride (151 to 311) and a low total nitrogen (6 to 28). Here again, it can always be demonstrated that the flows showing the lower chloride were determined earlier in the experimental

[2] The results on the fish in the oil-cloth car indicate urinary flows higher than those of the non-oil-cloth car. It must be considered that these fish had been fasted for 18 days. When we came to tie them up for urine flow, we realized that they were extremely active and much more difficult to handle than any we had encountered before. Our experience with this batch of fish made us more abrupt with the fish in the non-oil-cloth car with much less handling as a consequence. Furthermore, if there is any "excitement" or nervous factor in the diuresis, this batch of oil-cloth car fish would be the most likely to show it.

life of the fish or under better conditions (no scrubbing) than those showing the higher urinary chloride. It is interesting that the highest urinary chloride (340) so far recorded, when the flow was also obtained accompanied a flow of 17.1 cc. in a scrubbed fish. A fish in the same batch who died less than 48 hours after scrubbing showed a urinary chloride of 382. The highest flow showing zero chloride was 11.5 cc. (total nitrogen − 237).

In the early part of the investigation the data indicated that the urine flow steadily increased up to the death of the animal, but a careful study of many fish from the time of catching until their death demonstrated that without question the urine flow in by far the great majority of the fish examined rises to a peak and then diminishes as death approaches. The urinary chloride generally continues to increase in spite of the decreasing flow.

From the results indicated above, it appears that the normal flow of the sculpin is usually less than 4 cc. and that of the toadfish usually below 2.5 cc. per kgm. per 24 hours, when determined in the summer time when both species of fish are at the height of their activity. (Urine flows on the toadfish have been found to be normally markedly diminished in winter during the hibernation period.) However, we cannot convince ourselves that the normal flow of the sculpin may not be as high as 10 cc., because such flows have occasionally been found where the fish satisfied all of the present criteria for normality (no. 6, table 6; also see no. 3, table 4). But, on the other hand there is no proof that in the initial diuresis from injury all of our criteria for normality may not be present for a brief period (the chloride concentrations may be maintained normal by adaptations and the initial diuresis cause no lowering but an actual increase in the total nitrogen concentration in the urine (table 4)). Our results would appear to indicate that the urine flow of a glomerular fish may be greater than that of an aglomerular fish under normal conditions of existence. How fundamental such a difference is cannot be stated at the present time. When one considers capacity for urine flow, the highest flow recorded by us for the sculpin is 112 cc., for the toadfish, 68 cc. It is worth reiterating that the normal urine of toadfish and sculpin is undoubtedly almost invariably chloride free.

The second object of this investigation, to determine the factors responsible for the abnormally high flows frequently observed, we have accomplished only to the extent of demonstrating that skin injury is certainly a principal factor. Smith (1930) adduces conclusive evidence that marine fish swallow sea water, and in view of his findings, it seems reasonable to assume that the diuresis occurring in sculpin and toadfish with handling is dependent upon increased swallowing of sea water, brought about in some manner by the skin injury. The constancy of the composition of the internal environment of the fish is abolished, the plasma chloride approaching that of sea water in amount.

The nature of the diuresis has already been discussed following table 3. In the early stages of the diuresis, the urinary chloride is low and the total nitrogen high, but these relationships are rapidly reversed. It is noteworthy that, if a fish is handled with even reasonable care, the chloride will almost invariably remain low on the day of catching, even though he be handled many times. It should also be stressed that, from our data, the water excretion and the chloride concentration in the urine do not run parallel.

SUMMARY

Evidence is presented from work on the sculpin and toadfish which indicates that the urine flow of marine teleosts under normal conditions of existence is quite low (below 4 cc. per kgm. per 24 hrs.), much lower than figures reported in the literature would lead one to believe. The normal urine of these two species has been shown to be almost invariably free from chloride and relatively high in total nitrogen. Injury to the skin has been identified as a principal factor in causing the abnormally high flows encountered under experimental conditions. Associated with the abnormally high urine flow is a marked rise in chloride concentration in plasma and urine. In the course of the diuresis, which typically increases to a peak, then decreases with approaching death, the low chloride and high total nitrogen concentrations present in the urine in the early stages are rapidly reversed.

In conclusion, I wish to express my sincere appreciation to Prof. E. K. Marshall, Jr., for suggesting this problem, and for his valuable advice and criticism throughout the investigation.

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VITAMIN A POTENCY OF RETINAL TISSUE[1]

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Although it is now recognized that a deficiency of vitamin A is accompanied by widespread changes in tissues other than the eye, the ocular manifestations appear so regularly and provide such an easily available means of diagnosis that they still are largely depended upon in studies of this food factor. The characteristic ophthalmia involves changes in the cornea, the surrounding membranes and the periocular glands (see Yudkin, 1922; Mori, 1922). However, other less obvious changes occur in the eye in the absence of vitamin A in the ration. Thus the development of hemeralopia has been shown by Holm (1925) to be an early symptom of this deficiency and Fredericia and Holm (1925) have demonstrated that under these nutritive conditions the regeneration of the visual purple after bleaching is markedly delayed. That metabolic disturbances are also set up in the eye in the absence of vitamin A is shown by the increase in lipoid material in the pigmented layer of the retina (Sugita, 1925). It thus appears that in addition to the more familiar keratosis, certain functional changes of considerable importance occur in the eye as a result of a deficiency in this food factor.

As a preliminary study to the examination of the changes in the retina and choroid resulting from deprivation of vitamin A, the content of this factor in these tissues was determined. Holm (1929) has shown that the retina of calves' eyes will cure ophthalmia in rats in quantities of 750 mgm. of fresh tissue. Satisfactory growth was also induced by this amount of retinal tissue though no attempt was made to provide vitamin D in the basal ration. In the present study the experimental technique has been refined and an effort made to determine the potency of the retina within narrower limits.

PREPARATION AND ANALYSIS OF THE OCULAR TISSUE. Pigs' eyes brought

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direct from the slaughter house were freed from obvious surrounding fat. They were then cut with a razor blade, the fluids discarded, the retinae removed by fine forceps and transferred to cold distilled water. By careful dissection and washing with water much of the adhering choroidal pigment was removed. The retinal tissue was then transferred to a petri dish and quickly frozen on a block of solid carbon-dioxide. The frozen tissue was placed in a Hempel desiccator and dried in vacuo under sulfuric acid. The dried retina was ground in a mortar and stored in a brown bottle under carbon dioxide gas in a refrigerator.

The choroid layer was prepared in the same way except that the washing in cold water before freezing was omitted.

Approximately 1 gram of dried retina was obtained from 100 eyes. The material as fed contained 5.9 per cent moisture, 20.3 per cent ether extract, 18.1 per cent hot absolute alcohol extract and 12.2 per cent nitrogen. The alcohol extract was obtained from the residue of the ether extraction; both extracts were yellow in color. The arsenic trichloride test (Rosenheim and Drummond, 1925) was strongly positive with the ether soluble material but negative with the alcohol extract.

METHOD OF BIOLOGICAL ASSAY. Both male and female rats were used. They were started on the experiment at 21 days of age, and were kept in individual metal cages with raised bottoms. Definite signs of ophthalmia appeared in approximately 30 days in the animals on the curative experiments receiving vitamin A free-basal diet only. They were then given weighed quantities of the supplementary material (retina or choroid) which was readily consumed in every case. In the preventive experiments the supplements were given from the beginning. The basal ration consisted of casein (extracted with alcohol and ether and then heated at 100° for 24 hours) 18 per cent, corn starch 63 per cent, Crisco 15 per cent, salt mixture (Osborne and Mendel, 1917) 4 per cent. Vitamins B, D and G were provided by 400 mgm. irradiated dry yeast daily fed apart from the basal ration. Distilled water was freely available. The efficacy of the supplement as a source of vitamin A was judged by 1, cure or prevention of ophthalmia; 2, resumption or maintenance of increase in body weight, and 3, absence of renal and vesicular calculi, and of abscesses at base of the tongue and in the middle ear at autopsy. Food intake and body weight were recorded.

RESULTS. The results are summarized in the accompanying table and in the growth records represented graphically in the chart. That the dried retina is a potent source of vitamin A is shown by the fact that in the 6 animals given 50 mgm. per diem and in the 6 given 30 mgm. of this material after the development of ocular symptoms, the eyes were restored to normal appearance in 3 to 7 days and in 5 to 11 days respectively. Growth at the same rate as that of the positive control animals was induced

TABLE 1

Retina and choroid as sources of vitamin A

SUPPLEMENT	NUMBER OF ANIMALS		AVERAGE DAILY FOOD INTAKE		AVERAGE GAIN IN BODY WEIGHT PER DAY IN EXPERIMENTAL PERIOD		TIME ELAPSED BEFORE EYES CURED	NUMBER OF ANIMALS WITH ABNORMAL EYES AT END OF EXPERIMENT	NUMBER OF DEATHS	AUTOPSY	
										Number of animals with abnormalities	Organs found abnormal
	♂	♀	♂	♀	♂	♀					
			grams	*grams*	*grams*	*grams*	*days*				
Curative experiments:											
50 mgm. retina	2	4	9.9	8.1	2.7	1.5	3–7	None	None	None	None
30 mgm. retina	3	3	8.4	7.5	1.5	1.3	5–11	None	None	None	None
20 mgm. retina	3	2	8.9	7.9	1.0	1.0	5–21	1	None	2	Teeth, tongue, eyes, lungs
50 mgm. choroid	3	1	6.2	6.3	0.5	0.6	Not cured	4	2	1‡	Tongue, eyes lungs
100 mgm. butterfat	1		9.5		1.4		5	None	None	None	
Normal*					2.4	1.2					
Preventive experiments:											
20 mgm. retina	1	3	8.0	7.3	2.4	1.8		None	None	None	None
100 mgm. butterfat	2	2	8.4	7.1	2.6	1.9		None	None	None	None
No supplement	1	2	2.4	3.3	−2.7	−2.2	Not cured	3	3	3	Tongue, eyes, Harderian gland, ear
Normal†					3.8	2.3					

* From data on growth from 55 to 115 days of age, Smith and Bing (1928).

† From data on growth from 21 to 85 days of age, Smith and Bing (1928).

‡ The two rats which died were not autopsied.

by this quantity of retinal tissue. Furthermore none of the abnormalities usually attributed to a deficiency of vitamin A were detected on autopsy; the rats were in a thrifty condition and showed considerable amounts of intra-abdominal and subcutaneous fat.

Five animals were given 20 mgm. dried retina daily in the curative experiments. In 2 of these rats the ocular symptoms were cleared up within 5 and 7 days respectively; in two others, in 20 and 21 days; while the eyes

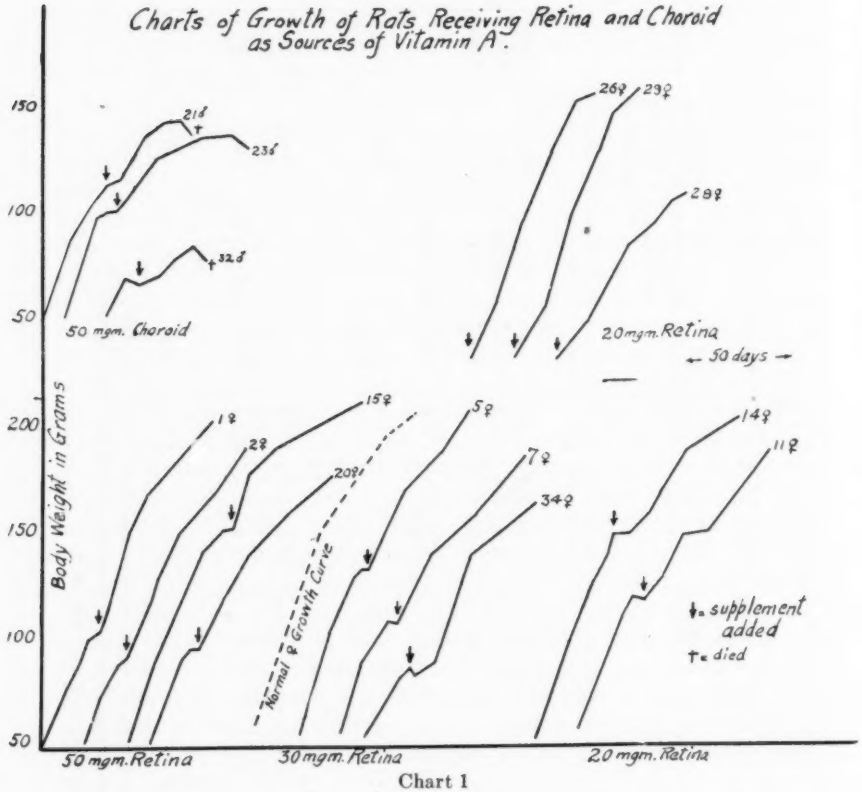

Chart 1

of the fifth rat remained abnormal throughout the experimental period. In two animals of this group typical lesions were found at autopsy. Atelectatic areas in the lungs were frequently encountered in rats which had been subjected to a lack of vitamin A but are not considered characteristic of this deficiency (see Moise and Smith, 1930). Resumption of growth was induced by 20 mgm. retina a day but at a somewhat slower rate than that of the control animals.

The inability to completely free the retina from the pigmented choroid coat necessitated the assay of the latter for vitamin A. Four animals were given 50 mgm. choroid as a curative measure. In no case were the ocular symptoms improved; two rats died at 31 and 41 days respectively after the appearance of ophthalmia but were not autopsied. The third showed, in addition to the ocular manifestations, pus in the lingual glands, while both this and the fourth rat had little intra-abdominal and subcutaneous fat. Leg weakness or posterior paralysis occurred in the animals of this group. The body weight of this group of rats increased very slowly throughout the experiment. It is thus obvious that 50 mgm. of choroid possessed little, if any, vitamin A potency when measured under these conditions.

It is expected that less vitamin-containing material will be required to prevent the appearance of symptoms of deficiency than to cure the condition, once it is established. Twenty milligrams of retinal tissue were given to 4 rats from the beginning of an experiment. Over a period of 59 days the average daily gain of 1.8 grams per day for the females compares favorably with 1.9 grams gained by animals receiving 100 mgm. butter fat; similarly the male rat gained 2.4 grams against 2.6 grams gained by the control. In no case was there any evidence of abnormality at autopsy.

Discussion. The foregoing observations on the vitamin A potency of the retina of hogs' eyes confirm those of Holm (1929) with calves' retinae. The potency of the retina is not augmented by the unavoidable contaminating traces of choroid, though the fact that the animals receiving choroid did not lose weight as did the negative control rats, indicates that this tissue as fed may have included some retinal tissue. This might be expected from the close, anatomical relationships of the two structures.

The arsenic trichloride reaction was positive for the ether extract which made up one-fifth of the dried retina; the remaining lipoid material soluble in absolute alcohol gave a negative test. If this reaction is indicative of the presence of vitamin A or its precursor we have in the present experiment a demonstration that the vitamin A potency of 4 mgm. of ether-soluble lipoid from the retina is sufficient for normal growth and function in the young rat. It thus appears that the retina is one of the richest known sources of this food factor.

It is not usual to find a localization of a given food factor in the tissue which gives evidence of a general deficiency of that factor. The characteristic keratosis of the cornea and epithelial structure of the periocular glands in the absence of vitamin A seems to be a typical response of protective and glandular epithelium to this deficiency. The delay in regeneration of visual purple after bleaching observed by Fredericia and Holm (1925) in rats deprived of vitamin A may be a result of altered metabolism of the cells of the retina under this dietary stress, as may also be the concomitant hemeralopia. These facts may be harmonized with existing

views of the physiology of vitamins and still not explain the high concentration of vitamin A in the retina. However, the recent studies of v. Euler and his collaborators (1928), Capper (1930), Green and Mellanby (1930) and Moore (1929, 1930) in which a generic relationship between the natural pigment carotene and vitamin A has been strongly implied suggest that the vitamin A (or its precursor) localized in the retina may bear a more or less direct structural relationship to the retinal pigment. This suggestion is given added weight by the observation of Holm (1929) that both white and gray matter of the brain is a poor source of this food factor.

SUMMARY

The retina has been shown to be a rich source of vitamin A. Thirty to 50 mgm. of dried normal retinal tissue per day will alleviate the characteristic symptoms of a deficiency of vitamin A in young rats. When given from the beginning with a ration deficient in vitamin A, 20 mgm. of dried retina will promote normal growth and physiological well being in young rats. The choroid contains little, if any, vitamin A.

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STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

XXX. Control of the Special Secretion of the Crop-Gland in Pigeons by an Anterior Pituitary Hormone

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The anterior pituitary is the known source of two or three hormones whose separation and identification have not yet been accomplished in a completely satisfactory manner. Hormones from this gland have already been rather definitely associated with several different reactions or processes in the organism. Another wholly distinct reaction of an anterior pituitary hormone is described here.

Pigeons of both sexes brood or "incubate" their eggs. When young are hatched the parents feed them, by regurgitation from the crop (*ingluvies*), a partially digested food which is plentifully mixed with a secretion and sloughing of mucosa cells—their so-called "crop milk." In preparation for providing this milk the mucosa of the crop, in both sexes, undergoes a most extraordinary growth and development during the last two or three days of the incubation period. It thus results that as soon as the young are hatched—and only then and for a short time thereafter—the parent birds develop, in two restricted dorso-lateral areas of their crops, two very elaborate temporary structures which produce "crop-milk."

It is the purpose of this paper to show that this special and rhythmic development of these special glands—coming at the end of the egg-brooding period and preparing the brooding parents for a "feeding-of-young" period—is controlled by a hormone produced in the anterior lobe of the pituitary. Also, to demonstrate that this reaction of crop-gland tissue may be and must be used in making a complete inventory of the hormones and hormone actions of the anterior pituitary.

Experimental results. In connection with some earlier studies (Riddle and Flemion, 1928, and unpublished data) on the effects of the "sex maturity" hormone in immature doves a few cases of enlarged crop-glands were observed, though not reported. As then observed, after prolonged injection of an anterior lobe extract (Lang), this result was too inconstant (due, we now believe, to a too prolonged period of dosage—7 to 65 days) to permit a definite conclusion. Other studies in this

laboratory during recent years have shown definitely that several other substances and hormones—insulin, adrenalin, folliculin, desiccated thyroid and thymus—do not produce a similar growth and functional response in these crop-glands. The tests recorded in this paper were

TABLE 1

Summary of tests showing the presence of the crop-gland stimulator in the anterior pituitary

The 33 birds serving as control—after daily blank injections (25 doves and 8 pigeons), all with normal crop glands—are not tabulated.

HORMONE OR EXTRACT	AMOUNT INJECTED DAILY	DURATION OF DOSAGE	BIRDS USED		NUMBER ENLARGED OR FUNCTIONAL CROP-GLANDS	NUMBER RAT UNITS USED PER BIRD
			Kind	Number		
Anterior lobe extracts						
	cc.	*days*				
Commercial (Lang)	0.3	3–11	Doves	12	12	
	0.4	2–3	Pigeons	2	2	
Growth hormone*	0.2–0.4	2–9	Doves	5	5	
	0.8	4–6	Pigeons	4	4	
Sex maturity*	0.4	4–5	Doves	2	2	
	0.4–0.8	4–6	Pigeons	4	4	
Antuitrin	0.1–0.5	3–5	Doves	5	0	
	0.7	3–4	Pigeons	3	0	
Posterior lobe extracts						
Pitocin	0.1	3	Doves	2	0	
	0.1–0.5	3–5	Pigeons	3	0	
Pitressin	0.1	3–4	Doves	2	0	
	0.1–0.5	3–5	Pigeons	3	0	
"Luteinizing" hormone, from pregnant urine						
Luteinizing*	0.4	4–5	Doves	5	0	70–90
	0.8	4–5	Pigeons	4	0	160–200

* Fresh extracts supplied through the courtesy of the Research Laboratory of Parke, Davis & Company.

therefore recently undertaken with a view to learning, *a*, whether definite and constant responses are obtainable from pituitary extracts, and *b*, something further concerning the specific source of the hormone involved.

The data of table 1 supply several of the essential facts. It is here

necessary, however, to describe the preparations and the birds used in this study. All the birds were killed for examination at about 75 days after hatching, with exception of two extremely young doves killed 2 and 3 weeks after hatching. This made it certain that none of these birds could normally show a development and functioning of the crop-glands—since this occurs only after sexual maturity, and then only at the end of a period spent in the incubation of eggs.

Of the 29 birds treated with an extract which contains the necessary principle all showed a clear response in the crop-glands; 16 of these were males, 13 were females. Other birds, totaling 27 of those of table 1, received other extracts which in no case elicited a crop-gland response. In addition 33 control birds which had been given blank-injections were used; none of these showed any trace of crop response. The normal weight of the resting dove mucosa—the portion that responds to pituitary stimulus—is about 120 mgm.; the same fully functional gland, including part of the secretion on its surface, has a weight of about 1.800 grams.

The commercial extract of Lang is dissolved in glycerin, and the control birds for this particular series of tests were injected with glycerin only. It is of course unfortunate that we can not know the details of the procedure used in the preparation of this extract. From previous and present experience and other opportunities we are certain, however, that this extract is prepared from fresh bovine anterior pituitaries, and that it contains the gonad stimulating hormone in very large amounts; also, that it is highly irritating to bird tissues when given subcutaneously or intramuscularly; it was used here intraperitoneally only. The "growth" (from anterior lobe of cattle), "sex maturity" (from whole pituitary of sheep) and "luteinizing" (from pregnant urine) hormones were prepared for us by the Research Laboratories of Parke, Davis and Company. The method used in preparing the growth principle is essentially that of Putnam, Teel and Benedict (1928, 1929), slightly modified by Bugbee, Simond and Grimes (1931). The sex principle was also an alkaline extract obtained by methods described by Bugbee, Simond and Grimes, and rather similar to those employed by Evans and Simpson (1928) and others for the extraction of the growth hormone. It is probable that the growth hormone extracts used by us were contaminated with more or less of the maturity principle, and *vice versa*. One preparation (094721A) of the growth hormone was a weak stimulator of growth, another (094698) gave better growth; the weaker preparation nevertheless gave evidence of stimulation of the sex glands. After heating for 13 hours at 99°C. (known to destroy the growth principle) the preparation 094698 no longer had any power to stimulate the crop-glands; this was shown in 4 birds, each of which received 1.0 cc. daily during 5 days. The maturity hormone extracts markedly stimulated gonad development.

The luteinizing hormone (definitely standardized, see table) produced luteinization in bird ovaries *but was wholly without effect as a stimulator of development in the crop-gland.* This is a really important fact; for, even if it should be proved that this hormone has its origin in the anterior pituitary, it is nevertheless *clearly and entirely distinct from the one which evokes growth in the crop-glands.* Furthermore, this luteinizing hormone, in contrast to the growth and maturity hormones, produced no gonad or oviducal growth whatever; indeed it gave some evidence of reducing the size of these glands. Data supporting these several statements concerning effects of these several hormones on various organ weights will be presented soon by Riddle and Polhemus (1931).

The two antuitrin samples tested by us were fresh preparations, which were curiously but definitely without effect on the crop-glands. The tests made with the two active principles of the posterior pituitary—pitocin (alpha-hypophamine) and pitressin (beta-hypophamine)—proved entirely negative. This result with these two highly purified and standardized preparations clearly demonstrates that no contamination by these posterior lobe hormones can account for the results obtained with the anterior lobe extracts. All extracts (except Lang's) were injected into the pectoral muscles.

The difference in histological appearance of the normal and treated crop-gland areas is shown by the various microphotographs—all made with the same magnification. The earlier stages of response in the treated glands (fig. 4; compare fig. 3) is characterized by a rapid proliferation of the cells of the mucosa, and by the formation of numerous primary and secondary foldings in the mucosa. These new cells stain deeply (hematoxylin-eosin) with hematoxylin, while those of the resting mucosa fix only slight amounts of the stain. In later stages the secondary folds and proliferations may so multiply (fig. 2; compare fig. 1) as to permit only a part of an original fold to be included within the visual field. The final and functional stage of the mucosa includes further the formation and

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Explanation of figures. The mucosa of the pigeon's crop (its inner proliferating surface facing *left* in all cases except fig. 5)—in the special area capable of enlargement—is shown in all figures; only part or none of the muscularis or serosa is shown.

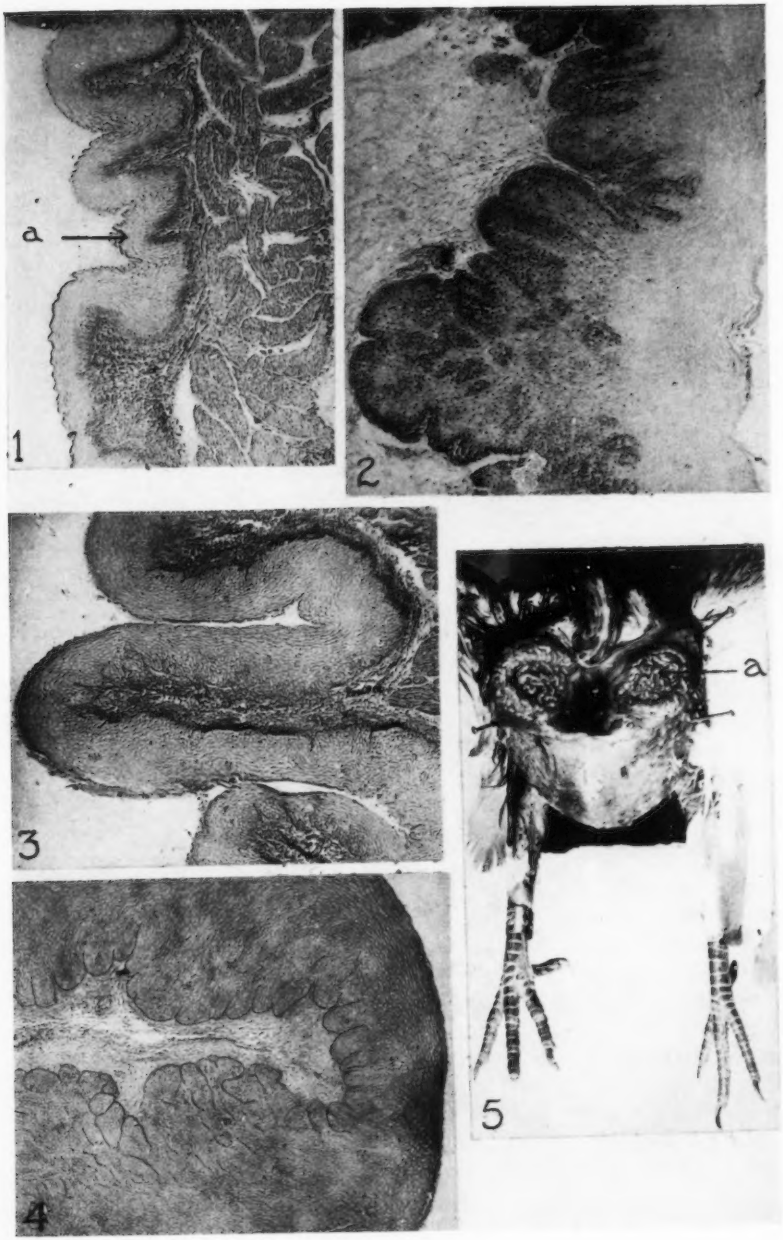
Fig. 1. Ring dove, 77 days old, control. Mucosa at *a*.

Fig. 2. Ring dove, 72 days old, 72 hours after beginning injection with highly potent sex maturity hormone from anterior pituitary.

Fig. 3. Common pigeon, 70 days, control.

Fig. 4. Common pigeon, 73 days, killed 52 hours after beginning injection with potent sex maturity extract of anterior pituitary.

Fig. 5. Entire crop area of ring dove with left crop denervated. Later injected during 7 days with growth hormone; from fourth to seventh day also with sex-maturity hormone. Only the region (on both sides) indicated by the arrow, *a*, has undergone enlargement.



Figs. 1–5

separation of the caseous masses of desquamative cells which largely compose this "crop-milk." These cheesy masses are entirely washed away and lost from tissues prepared for microscopic study, and largely so from glands prepared merely for weighing on a balance. The general appearance of the two fully functional glands, minus nearly all of these cellular masses, may be seen *in situ*, about one-half natural size, in figure 5.

It must be understood that only a fraction of the total inner area of the crop takes any part in the response to this hormone; this response, exactly like that which appears normally at the end of the incubation period in pigeons (Teichmann, 1889; Dulzetto, 1928), is confined to two dorsal or dorsō-lateral areas, each of about 3 or 4 sq. cm. in a dove. The beginning of the growth response is plainly visible, even macroscopically, only 48 hours after a first injection; after 4 to 5 days this responsive mucosa has changed from a paper-thinness to a bulky functioning mass. Our data suggest that long-continued light dosage almost certainly fails, and such heavier dosage probably fails, to maintain this high state of activity during many weeks or months. Possibly the mucosa of this area thus becomes temporarily exhausted, but this aspect of the subject requires further study.

It is to be noted further that this response of the crop-gland has been obtained in nestling doves—dosed from one week after hatching to the end of the second week. One such small fledgling, still receiving "pigeon-milk" from its parents, had its own glands fully activated to the production of its own "pigeon-milk"—and so far as this gland was concerned this infantile dove was fully prepared for the parental act of feeding young. Another fledgling bird, at the end of its third week in life, was sacrificed and proved also to have fully functioning crop-glands. Palpation of the crop areas of three additional young, similarly treated between the beginning and the end of their second week, has assured us that these birds also then had much enlarged crop-glands. These three birds were not killed for visual examination but were continued in another study. The growth hormone (proved to contain also the maturity principle) was used exclusively in these tests on very young squabs.

We have tested the possibility that the anterior pituitary substance responsible for this growth response of the crop-gland may exercise its effects through the nerves of the crop. In two ring doves, at 70 days after hatching, we cut the nerve supply to the left crop-gland, leaving intact the nerves to the right side of the crop. This procedure was necessary because *a*, the nerve supply (N. recurrens) is most easily and surely severed by vagotomy at a higher level, where the N. esophagus (possibly anastomosing with N. recurrens near the crop, Ihnen, 1928) may also be found and cut; and *b*, the crop is thus not completely immobilized and the animal can be kept in suitable living condition. Any

possible nerve supply entering the crop-wall by way of the skin was excluded by a temporary complete detachment of the whole of the left crop region from all adjacent tissue. Immediately after this operation the two birds were injected with anterior pituitary hormone. One of the two birds died 3–15 hours after the operation. The other bird received 7 injections of growth hormone, and from the 4th to the 7th day some sex maturity hormone in addition, and was then killed for examination. Both the *left* and the right crop-gland areas made full response to the injection (fig. 5). The left gland is shown in the photograph to involve a slightly smaller area of enlargement—though equally and fully functional—probably because of the shrinkage of the whole of the left crop region due to lack of innervation and interference with its blood supply. The response of this area to the effective anterior lobe hormone is not mediated by the nerves.

Discussion. In view of other facts now known the tests described here make it quite clear that the response of the crop-gland of the pigeon is initiated by an anterior pituitary substance; also, that the luteinizing principle from pregnant urine wholly lacks this activating principle. Our data further provide the most cogent evidence that this luteinizing hormone also wholly lacks the power to stimulate gonad growth in doves and pigeons, and that the luteinizing and sex maturity principles are probably wholly distinct. Much of this evidence can be given only through a full presentation of gonad and other organ weights, and this will be done in another communication. It is of course not certain that the hormone obtained from pregnant urine is of pituitary origin.

It is also obvious that we do not now know whether this response of the crop is under the control of the growth, the sex maturity, or of a third and wholly unrecognized principle of the anterior pituitary. Every effective crop stimulant used by us has shown also gonad-stimulating properties. One preparation of the growth principle was perhaps weak in both growth and gonad-stimulating power, but still effective in evoking a crop-gland response. If this crop response is a specific response to the growth hormone it is the quickest and easiest method yet found for detecting this hormone. If this is only another action of the sex maturity hormone it is very singular that this crop area does not respond in females at the same time as does the ovary (i.e., just preceding ovulation), but at about two weeks later; and then only if brooding behavior has been continuously observed. It would be still more singular if the pituitary prepared a special hormone for the accommodation of those very few families of birds which secrete "crop-milk" and feed their young by regurgitation.

Among the hitherto observed responses to anterior pituitary hormones that of the mammary glands probably more nearly simulates the crop

response. The mammary and crop areas are of course not homologous structures, and perform merely analogous functions, but both represent *a*, phases of reproduction which are consequent to ovulation; and *b*, both responses occur only after a considerable interim following ovulation, and *c*, near to the time of a new phase in the alimentation of the young. Within the last three years several observers have found that an anterior pituitary hormone is concerned in lactation, but there is as yet no agreement on the question whether a corpus luteum or other ovarian factor is also involved in this mammary stimulation. If mammary and crop are stimulated by the same hormone, the present data demonstrate that corpus or other ovarian factors are not involved since the response is as immediate and complete in males as in females.

It should be added that in the practical use of the crop-gland for standardization or other tests it would be possible to use non-brooding doves or pigeons of any age and sex. Moreover, in the case of birds aged more than one month the animal need not be killed for examination; it would suffice to open the crop on its ventral surface, empty it, inspect the gland, and repair the crop-wall and skin with a few sutures.

CONCLUSIONS

Two dorso-lateral areas of the crop (*ingluvies*) of doves and pigeons normally undergo an extreme enlargement at the end of the brooding period and then secrete the "crop-milk" which is fed to the young. This complete glandular enlargement and functioning can invariably be induced in very immature and other non-brooding birds of both sexes by suitable extracts of the anterior pituitary and by such extracts only.

This growth response in the crop-gland area occurs after previous section of its nerve supply and is therefore not regulated or conditioned by the nerves.

All of the several effective extracts used were known to contain the sex maturity hormone in greater or less amounts; all may also have contained small amounts of the growth principle; all caused growth in the gonad and enlargement of thyroid and liver. The effective crop-gland stimulant is destroyed by heat.

The "luteinizing" hormone, as obtained from pregnant urine, is entirely ineffective in eliciting this response in the crop-gland. This result, together with no effects or possibly with adverse effects on gonad growth (to be more fully considered elsewhere) makes it quite improbable that the luteinizing and maturity hormones are identical or even closely related substances.

It is not now known whether the principle activating the crop-gland is the growth, the sex maturity, or a third and now unknown anterior pituitary hormone. Certain aspects of this problem are discussed.

If this response of this special part of the crop mucosa of the pigeon is a

specific response to the growth hormone, it provides the easiest and quickest known method of testing and standardizing that hormone. A clear macroscopic result is obtained in 72 hours, and active "milk" secretion occurs on the fourth or fifth day.

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EFFECT OF DIFFERENT PER CENTS OF PROTEIN IN THE DIET

VI. Weight of Mothers during Gestation and Lactation[1]

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Making use of five groups of rats, I, II, III, IV, and V, and feeding them continuously on carefully prepared synthetic diets containing 10, 14, 18, 22 and 26 per cent of protein respectively we have previously given the results of the effect on growth, spontaneous activity, food consumption, reproduction and the offspring (Slonaker, 1931a, b, c, d, e). In this paper we propose to give the results of the effect of these diets on the gestation and lactation weights of those mothers whose weights were taken daily during these times.

Rowe, Gallivan and Mathews (1930) in regard to metabolism during pregnancy of women state

> pregnancy produces an increase in the basal rate, during the last twenty-four weeks, of 13 per cent more than predicted from the change in weight. Following delivery there is a downward trend of significant proportions which reaches a minimum value somewhere between the third and fifth week, and then rapidly rises to a level within the normal zone. With the exception of the post-partum depression, the average deviation values fall within conventional normal limits.

The last twenty-four weeks of human gestation are equivalent to from the seventh or eighth day of gestation to parturition in the rat, and the third to fifth week to the second or third day in lactation. We have not tested the rat to see if the same conditions obtain. The results of Magnus-Levy (1904) given by Lusk (1928) show an increased oxygen requirement on the part of the mother as pregnancy advances. It has been shown (Rubner, 1908) that the weight of the mammalian embryo as compared to that of the mother is almost unappreciable until about the middle of the gestation period and that up to this time the mother shows little of any change in metabolism (Zuntz, 1908). "At term, however, the weight of the child is between 5 and 6 per cent that of the mother, and when the various ad-

[1] This research has been assisted by the Department of Physiology, the National Research Council through its Committee for Research in Problems of Sex, the National Live Stock and Meat Board, the Fleischmann Company and their successors, the Standard Brands, Inc., and the coöperation of Albers Bros. Milling Company.

nexa are considered the mother loses during parturition the equivalent of nearly 20 per cent of her postpartum weight" (Lusk, 1928). The results of Carpenter and Murlin (1911) given by Lusk (1928), show that the average loss in weight of three women at parturition was 10.1 kgm. Of this the child averaged 3.1 kgm. and the adnexa 7 kgm. The weight of the child was thus 5.85 and the adnexa 13.14 per cent of the postpartum weight of the mother.

In a study of the effect on reproduction and lactation of different proportions of meat in a mixed diet on the rat MacLeod (1927) has shown that with the increase of the protein content of the diet from 9.9 to 17.5 per cent by the addition of raw meat, and from 9.1 to 14.9 per cent by the addition of dried meat beneficial results were obtained in both reproduction and lactation. She found that a greater number of young of heavier weight were reared on the diets containing the larger per cents of protein. This indicates that the mothers were able to furnish a better supply of milk to the nursing young. The weights of the mothers during gestation and lactation were not given.

Drinkard (1917) investigated the effects of high protein and high energy rations on feeding dairy cows. He used two types of rations, one having a nutritive ratio of 1:11 and the other 1:24. On the high protein diet the cows consumed only three-fourths of the ration, but this was sufficient for the energy requirements and over twice the protein needs. On the high energy and low protein diet the animals lost weight during lactation due to a lack of digestibility and protein starvation. The coefficients of digestibility of all the nutrients were reduced to a remarkable degree. Much better milk production was attained on the high protein diet.

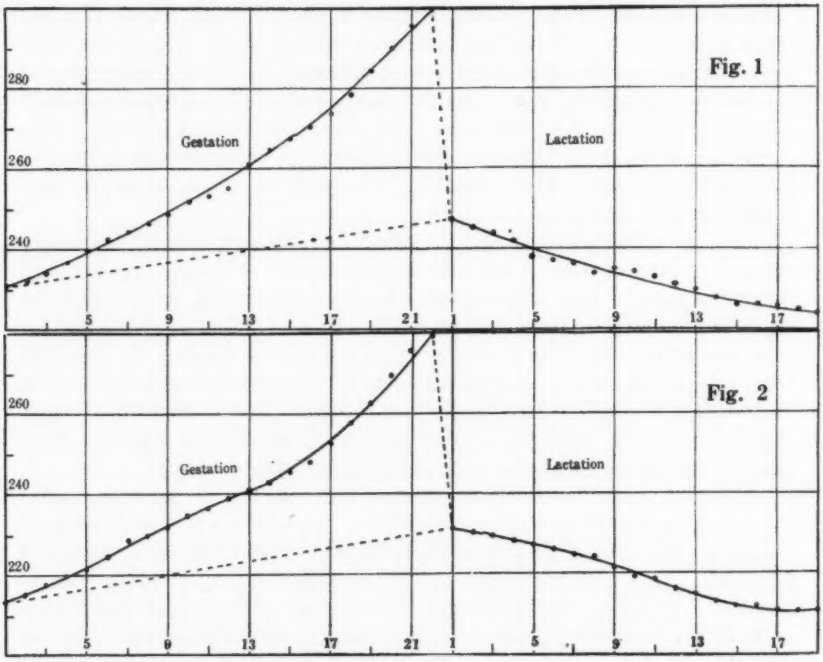
Hitchcock (1926) studied the effect of meat feeding on nursing mother rats and the rate of growth of their litters. In addition to a well balanced diet 8 to 10 grams of meat were given daily to the mothers from the day of delivery. As the young began to eat the amount of meat given was increased. In his summary he states "It is shown in this paper that female rats that were nursing litters weighed more and raised larger and healthier litters when they were fed meat in addition to an adequate balanced ration. The rate of growth of the newborn rats was faster when their mothers received meat."

In an investigation on the relation of diet to reproduction and lactation Wilkinson and Nelson (1930) used three kinds of soy bean in amounts ranging from 10 to 73.3 per cent and found that satisfactory lactation was not obtained on any level of the soy bean content, but on addition of liver, kidney, pancreas, brain and heart there was improvement. This indicates that the quality of the protein is essential.

The above citations are sufficient to show that normal reproduction is dependent on the quantity and quality of the diet given and that certain

changes in body weight and metabolic rate are closely associated. They also indicate that a rat may be able to give birth to young when fed a certain diet and yet not be able to rear them. They further show that the best growth of the offspring depends in a large measure on the quantity and quality of the protein constituent in the diet.

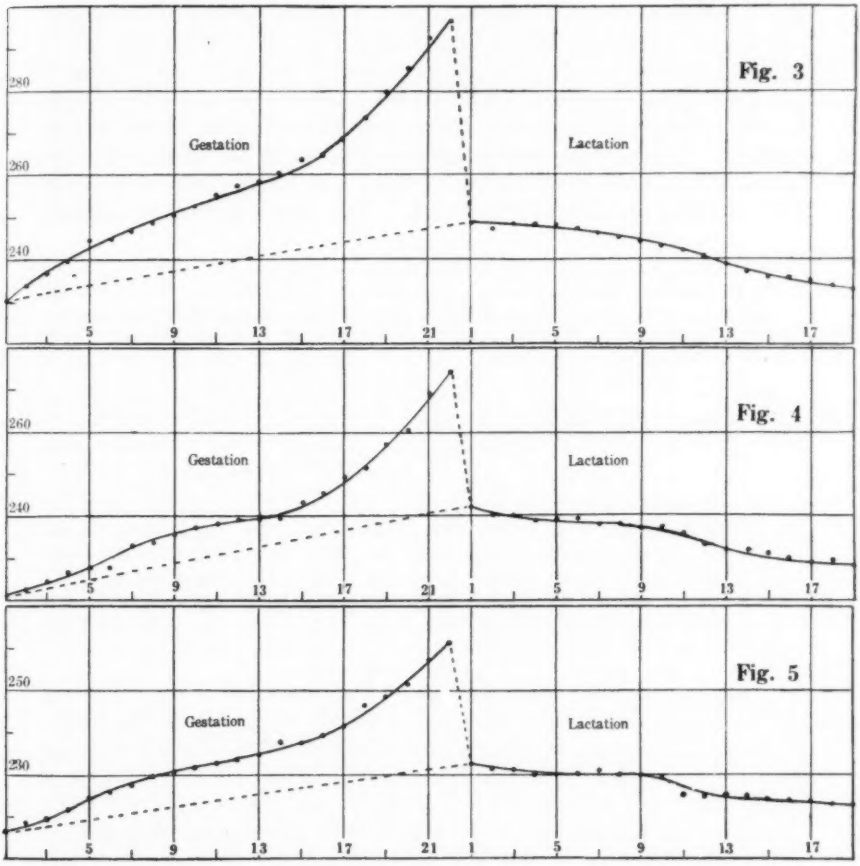
In our experiment the mothers were weighed daily from coitus to the day the young were weaned and the average weights on successive days determined for each group. In figures 1 to 5 are shown the curves of these



Figs. 1 and 2

average weights from the day of coitus to the nineteenth day of lactation for each group from I to V respectively. The hypothetical growth curve of the mother is indicated by the dotted line extending from the weight at coitus to the first weight following parturition. We have no way of ascertaining how closely this approaches the true curve of normal growth. Attention has been previously called to the great reduction of spontaneous activity in female rats during gestation and lactation accompanied by an increased food consumption (Wang, 1923; Slonaker, 1925). During ges-

tation the increased food intake was relatively small and almost uniform in amount, but during lactation it increased rapidly as the young became older. It is quite possible, therefore, in view of the results cited above, that the increase in weight shown for the first few days of gestation was



Figs. 3 to 5

In each figure the ordinates represent the average body weight in grams; the abscissas, days in gestation and in lactation. The abrupt drop in the curve, shown by dotted line, indicates the birth of young. The dotted line from weight at coitus to the first postpartum weight indicates the increase in body weight due to normal growth.

Figures 1, 2, 3, 4 and 5 represent the average daily weights of groups I, II, III, IV and V respectively during gestation and the first 19 days of lactation.

due principally to an increased weight of the mother rather than to the developing young. A greater intake of energy and a less expenditure would indicate this occurred.

On comparing figures 1 to 5 one is impressed by their great similarity. The great augmentation in weight occurred during the last half of gestation and became more pronounced up to the day of delivery. The gestation weights of group I form a simple curve while those of III, IV and V form a compound curve. The curve of group II is a transition between I and III. These differences may have been partly due to a lack of conformity in spontaneous activity exhibited by these different groups during gestation. The average daily activity as indicated by the number of revolutions turned by each of the groups from I to V was 3443, 3148, 2860, 2670 and 3011 revolutions respectively. The more sedentary the animal

TABLE 1

Giving the number, average age in days, the weights in grams of mothers at coitus, at the twenty-second day of gestation and their gain in weight in the different groups

GROUP	NUMBER	AVERAGE AGE	AVERAGE SIZE OF LITTER	WEIGHT							
				At coitus	At 22nd day of gestation	Gain					
						Total	Normal growth	Developing young			
								Grams	Per cent	Per each young	
										Grams	Per cent
I	44	290	5.38	231	299	68	16	52	22.50	9.84	4.26
II	54	343	5.70	213	279	66	18	48	22.53	8.42	3.95
III	43	387	4.90	230	296	66	18	48	20.87	9.80	4.26
IV	24	444	4.95	221	274	53	21	32	14.49	6.47	2.93
V	21	494	3.95	216	261	45	16	29	13.43	7.34	3.39

the more favorable are the conditions for the deposition of adipose tissue (due to a relatively greater amount of available energy for this purpose). Since the weight of the developing young does not materially influence the weight of the mother for the first few days of gestation the early increase of weight shown in all the groups was doubtless due to deposition of fat and the differences in the curves to the rate at which this was accomplished.

The rate of increase in weight of the mother during the last part of gestation, and the maximum weight attained just before parturition depend on the number of young being developed. In table 1 we have given the average size of the litters born in each group. It is seen that the largest litters were in groups I and II and that the smallest was in group V. It is also seen that the grams and per cents gained due to the developing young were greatest in groups I, II, and III and least in group V. This was expected since these facts are in conformity with the size of the litters. The

gain per each young born, however, was not uniform in the different groups. Table 1 shows that the gain in weight of the mothers for each young born was largest in group I and least in group IV. This could not have been due to the young of group I being larger for, as shown in table 2, the young of group IV averaged the heaviest of any of the groups and were almost equaled by groups III and V. We have assumed that the difference between the weight of the young at birth and the loss of weight of the mother at parturition can be attributed to the weight of the birth adnexa. These weights for each young born are given in table 2. It is noted that there was a marked lack of conformity in these weights. Groups I, II and III showed relatively large weights while in groups IV and V they were small. The per cents of total loss attributable to birth adnexa are considerably below that of the human (13.14 per cent) cited above. In a study of the

TABLE 2

Giving the average age at parturition and the weights lost at delivery of the mothers of each group

GROUP	NUMBER	AVERAGE AGE	AVERAGE LITTER	WEIGHT											
						Loss at parturition									
						Total		Young				Birth adnexa			
								Total		Per 1 young		Total		Per 1 young	
				At 22nd day of gestation	After delivery	Grams	Per cent	Weight	Per cent	Weight	Per cent	Weight	Per cent	Weight	Per cent
I	44	312	5.38	299	247	52	21.2	27.9	11.3	5.19	1.92	24.1	9.9	4.47	1.81
II	54	365	5.70	279	231	48	20.8	29.4	12.7	5.16	2.23	18.6	8.1	3.26	1.41
III	43	409	4.90	296	248	48	19.3	26.6	10.7	5.42	2.18	21.4	8.6	4.38	1.77
IV	24	466	4.95	274	242	32	13.2	27.0	11.1	5.46	2.26	5.0	2.1	1.01	0.42
V	21	516	3.95	261	232	29	12.5	21.4	9.2	5.41	2.33	7.6	3.3	1.93	0.83

prenatal growth of the guinea pig Ibsen (1928) found that at full term the percentage distribution of the contents of the uterus was embryo 80.967; placenta, 3.982; fetal membranes, 1.935; decidua basalis, 0.534; amniotic fluid, 12.583. This shows that approximately 81 per cent was due to the young and 19 per cent to the birth adnexa. Making use of the data given by Warwick (1928) in his paper on prenatal growth of swine we find that the young weighed approximately 83 per cent and the adnexa 17 per cent of the uterine contents. These results are quite different from those found in the human where the fetus weighed about one-third and the adnexa two-thirds. The average of all groups of our rats gave an approximate distribution of young 64 per cent and adnexa 36 per cent. The difference between our results and those cited for the guinea pig and swine was very likely due in part at least to the cannibalistic habit of the rat. It is well

known that in the rat the mother eats the fetal membranes. The fluids incidental to birth are lost. Her postpartum weight would therefore be increased an amount equal to the weight of the fetal membranes. This, however, does not account for the marked differences in the weights of the adnexa in the different groups. The only explanation we have to suggest is that the mothers of groups IV and V may have eaten a greater number of their young at birth than the other groups which would result in a higher postpartum weight than normal.

Figures 1 to 5 show that the mothers lost weight during lactation in each of the groups. It is seen that in the different groups the amount lost and the rate of loss were not the same. Group I showed the greatest loss with almost a uniform daily loss. Group V had the least loss and the rate of loss was not constant. Groups II, III and IV showed gradual transition

TABLE 3

Giving the number of observations, the average age and weight at parturition, the average weight at the nineteenth day of lactation, the average and per cent lost and the loss per each young weaned in the different groups

GROUP	NUMBER OF CASES	AT PARTURITION		AT 19TH DAY OF LACTATION			NUMBER OF YOUNG		LOSS PER 1 YOUNG WEANED			
		Average age	Average weight	Average weight	Average grams lost	Per cent loss	Average born	Average weaned	Average grams	Average per cent	Computed for litter of 5	
											Average grams	Average per cent
I	67	317	208	185	23	11.0	6.14	5.65	4.07	1.95	3.78	1.82
II	76	297	215	195	20	9.3	6.26	5.31	3.75	1.74	3.30	1.53
III	33	350	243	228	15	6.2	5.91	4.67	3.21	1.32	3.27	1.35
IV	23	407	248	234	14	5.6	5.48	4.44	3.15	1.27	3.22	1.20
V	18	471	220	211	9	4.1	5.11	4.06	2.22	1.01	2.80	1.27

from group I to V. During the first 9 days group I lost 12 grams while group V lost only 2 grams. The loss in each of the groups from I to V for the first 9 days was 12, 10, 5, 5 and 2 grams respectively. This shows the increasing tendency of the mothers to maintain their weight as the per cent of protein in the diet increased. In table 3 are given the average grams and the per cents lost in the five groups by the nineteenth day of lactation. This shows a reduction from 23 grams and 11 per cent in group I to 9 grams and 4.1 per cent in group V. The average loss for each young nursed is also given. This also shows a reduction of from 4.07 grams and 1.95 per cent in group I to 2.22 grams and 1.01 per cent in group V.

The size of the litters nursed, however, must be taken into consideration. In general a mother nursing a large litter loses more weight than one nursing a small litter. Table 3 shows that the average size of the litter weaned

in group I was 5.65 and that the size became less and less as the per cents of protein in the diet increased and was reduced to 4.06 in group V. Making use of the loss given for each young which was nursed we have computed what the loss per young would have been if each of the groups had nursed litters of 5 young. This is given in table 3. This computation has changed the value of all the figures, but they still show the same general arrangement as given for the number of young which were actually nursed. To show that these computed figures are not far from correct we have determined the average loss of weight of the mother per one young nursed in five litters of five each in group V. This was 2.64 grams and 1.27 per cent. The average of two other litters of 6 and 7 each in the same group was 2.76 grams and 1.27 per cent. This shows that the computed grams lost in this group was a little too large, but that the per cent loss was approximately correct. These data show that the task of nursing was accomplished more and more advantageously as the per cents of protein in the diet increased from 10 per cent to 26 per cent. Since the effect on the growth of the young nursed was augmented in this same order (Slonaker, 1931e) we are led to infer that the quantity and quality of the milk supply increased in the same manner, and that the mothers were able to produce this supply with a less expenditure of energy as the protein content of the diet increased.

SUMMARY

1. The curve of average weights of the mother during gestation changed gradually from a simple curve in group I to a compound curve in group V. This was probably due to an increasing deposition of adipose tissue during the early part of gestation.

2. The maximal weight attained was correlated with the size of the litter born.

3. The average per cent gained by the mother due to developing young was II, 22.53; I, 22.50; III, 20.87; IV, 14.49; V, 13.43. For each young born the per cents were I, 9.84; III, 9.80; II, 8.42; V, 7.34; IV, 6.47.

4. The loss of weight of the mothers while nursing was greatest in group I and became less as the per cent of protein in the diet increased.

5. Data indicate that the quantity and quality of milk secretion was poorest in the group receiving 10 per cent protein and increased as the protein content of the diet increased.

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THE OXYGEN CONSUMPTION OF MUSCLES MADE NON-IRRITABLE BY SUGAR SOLUTIONS

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It was first shown by Overton (1902, 1904) that muscles soaked in isotonic sugar solutions become non-irritable after 15 minutes but recover when again immersed in Ringer's solution or any non-toxic solution containing sodium ions. During this treatment the muscles lose considerable amounts of electrolytes, chiefly potassium phosphate. It is supposed that all the electrolytes in the interspaces between the muscle fibres diffuse away together with some of the potassium phosphate from the interior (Urano, 1908; Fahr, 1908). Embden and Lange (1923) showed further that this sugar non-irritability is accompanied by a large increase in oxygen consumption and an increased loss of phosphoric acid, which they interpreted as the result of an increase of permeability of the muscle fibres.

My own interest in the effect of sugar solutions began during a study of the oxygen consumption of muscles under the influence of different agents causing contracture. Sugar does in fact cause a slight contracture of the muscles which is accompanied as usual by an increased oxygen consumption and an increased lactic acid concentration. This contracture, however, is an inconstant accompaniment of the changes which take place and its intensity is variable and bears no quantitative relation to the excess oxygen consumed. It is probably somewhat incidental, therefore, to the processes concerned, and is probably conditioned by the attainment of a certain critical relation between the K and Na ion balance around the muscle fibres.

Of perhaps more importance than the contracture is the reversible non-irritability which occurs after sugar treatment. It seems that this also is caused by an increase in the K/Na ratio outside the fibres. As has long been known, high concentrations of potassium in Ringer's solution lead to reversible non-irritability associated with an increased rate of oxygen consumption and, at least temporarily, an increased rate of loss of phosphoric acid from the muscle (Embden and Lange, 1923; Vogel, 1922). It has recently been shown by Horton (1930) that the spontaneous non-irritability described by Dulière and Horton (1929) is caused by an in-

creased K concentration. This condition is also accompanied by an increased oxygen consumption (Fenn, 1929) which is promptly decreased by Ringer's solution. Other agents causing a similar type of non-irritability were discussed by Horton and it is surprising that he neglected to include the non-irritability due to sugar in his study.

In the following paper some new observations are reported concerning the behavior of muscles in sugar solutions and sugar-salt mixtures. It was hoped in particular that the results would provide a key to the understanding of the normal mechanism for regulation of the metabolism in living cells. In sugar the polarization of the cell membrane is increased (if measured by the injury current) but in contractures it is decreased. In both cases the metabolism is increased. This suggests the hypothesis

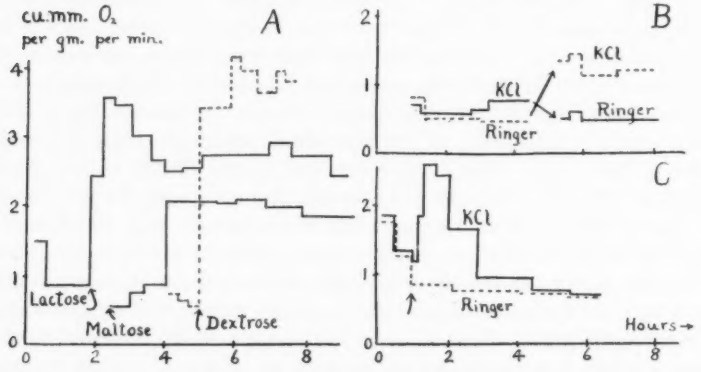

Fig. 1. The increased oxygen consumption of the frog sartorius muscle caused by isotonic sugar solutions and solutions of KCl. A. The effects of 6.8 per cent lactose, 6.3 per cent maltose, and 4.5 per cent dextrose. B and C. The effect of Ringer's solution compared to a solution of 0.2 per cent KCl and 0.65 per cent NaCl. B. Muscles removed from respirometer and exchanged. C. Solutions tipped in from a side arm.

that any deviation of the polarization from normal, either an increase or a decrease, causes a rise in metabolism. Such an hypothesis must however be more widely tested before it can be accepted as more than a working basis.

METHOD. The chief method used for this investigation has been the measurement of the oxygen consumption by means of the differential volumeter of Thunberg (cf. Fenn, 1927). The respiration chamber was provided with two side arms, one for holding NaOH to absorb CO_2 and one for holding various reagents such as isotonic sugar solution to be dumped upon the muscle at the proper time during the experiment. I

used chiefly the sartorius muscle of the frog (mostly *R. pipiens*) but not infrequently the ileofibularis or the semitendinosus muscles of the upper leg, both of which are small enough to permit of an adequate supply of oxygen by diffusion. The temperature was approximately 23 degrees in all cases and was controlled to within 0.01 degree in any one experiment. The muscles weighed between 60 and 150 mgm. The first experiments were done in September 1929 and have continued at all times of the year since then without there being any obvious seasonal correlation.

RESULTS. Some of the facts which have already been mentioned in the introduction are illustrated in figure 1. Three graphs are shown in figure 1A in which the large rise in oxygen consumption with isotonic solutions of lactose, maltose and dextrose is illustrated. The point where the sugar was tipped on to the muscle is shown by an arrow. Previous to the sugar treatment the muscle was in the dry respirometer bottle in oxygen but without Ringer's solution although it had been soaked in Ringer's solution after dissection.

The effect of increasing the potassium content of the solution to 0.2 per cent (in NaCl 0.65 per cent) is shown in figure 1B. The match muscle in Ringer's solution remained irritable while its mate in potassium showed a higher oxygen consumption and lost its irritability. On interchanging the muscles between the two solutions, as indicated by the arrows, their positions were reversed. The non-irritability and high oxygen consumption caused by potassium disappear on recovery in Ringer's solution. In such concentrations of potassium its effect is therefore reversible both as to oxygen consumption and as to irritability. Such a solution does not maintain the oxygen consumption at a high rate for more than about 4 hours.

This experiment partially illustrates the fact that *the oxygen consumption of muscles may be controlled experimentally by the K/Ca ratio* since Ca causes a fall (see fig. 5) while K causes a rise in metabolic rate. It should be mentioned, however, that the changes in Ca and K which are necessary for this purpose are far beyond the physiological range so that it is doubtful to what extent this mechanism operates in the body.

Figure 1C illustrates the effect of still stronger solutions of KCl. At the point marked by the arrow, isotonic KCl (0.9 per cent) was dumped on one muscle and Ringer's solution on the match muscle. This rise in oxygen consumption is due to the contraction produced by KCl and as I have shown elsewhere is of about the right order of magnitude to explain the tension-time which is simultaneously produced by the contraction (Fenn, 1931). The fall in oxygen occurring in this experiment immediately after the introduction of Ringer's solution is only a part of the progressive decrease in metabolic rate regularly found in these muscles when first dissected.

Similarity between sugar, potassium and spontaneous non-irritability. In muscles which have become spontaneously non-irritable through failure to soak them in Ringer's solution after dissection neither sugar nor potassium solutions cause the usual rise in the rate of oxygen consumption. This fact is shown by the graphs of figure 2 which represent the effects of both sucrose (6.5 per cent) and K_2SO_4 (1.3 per cent) upon the oxygen consumption of "washed" and "unwashed" muscles. The muscles after

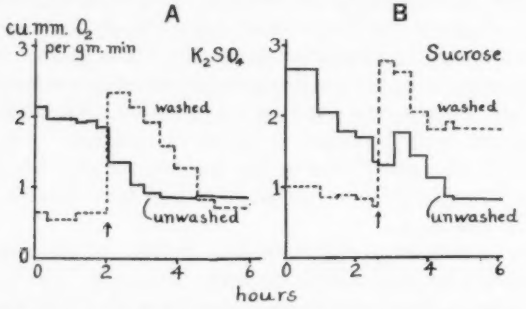

Fig. 2. The absence of an increase in the oxygen consumption in spontaneously non-irritable muscles (unwashed) when treated with 1.3 per cent K_2SO_4 and 6.5 per cent sucrose.

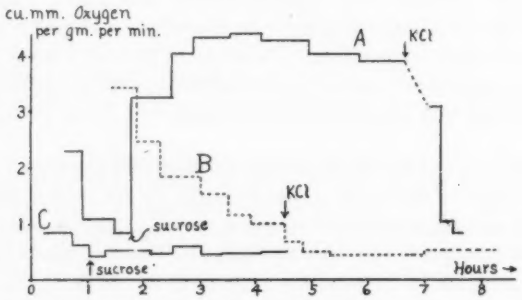

Fig. 3. A and B. Muscles made non-irritable by sugar give no increased oxygen consumption with 0.9 per cent KCl. C. A muscle made non-irritable by K_2SO_4 gives no increased oxygen consumption with 6.5 per cent sucrose.

preliminary treatment with or without Ringer's solution as the case may be, are put into the respirometer and remain there in oxygen only (not in solution) until the sugar or potassium solutions are dumped upon them. The unwashed non-irritable muscles show in both cases either an immediate fall in the oxygen consumption, as in the case of K_2SO_4 or a temporary and negligible rise followed by a fall in the case of sugar. Normal washed muscles, as indicated by the dotted lines show a prompt increase in oxygen

consumption in both cases. The failure of K_2SO_4 to produce the usual rise in oxygen consumption in unwashed muscles is matched by the fact that the contraction produced under such conditions is either absent altogether or much diminished, but returns after recovery in Ringer's solution.

It is likewise true that muscles which are non-irritable from sugar fail to show a rise in oxygen consumption from treatment with potassium solutions and vice versa. These facts are illustrated in figure 3. Curves A and B show the effects of KCl on muscles made non-irritable from sugar. In curve A the muscle is first in Ringer's solution. At the arrow, sugar is dumped upon it from a side arm and the oxygen consumption rises. At the second arrow the sugar solution is removed from the respirometer and replaced by isotonic KCl. The oxygen consumption which was previously at a rate of 4 cu. mm. per gram per minute is now promptly reduced to less than 1. In curve B the muscle was soaked in isotonic sucrose and was then removed from the sugar and put into the respirometer without being bathed in any solution. Readings were taken for 3 hours and at the point marked by the arrow isotonic KCl was introduced from the side arm of the apparatus. In this case the oxygen consumption had returned to normal limits after the rise due to the sugar but still KCl caused no second increase. A similar experiment with caffeine yielded a similar result although in a normal muscle this substance produces a fourfold increase in the rate of metabolism.

In experiment C (fig. 3) a muscle was made non-irritable by soaking in isotonic K_2SO_4 for half an hour. It was then removed from the solution and put into a respirometer. After some preliminary readings an isotonic sugar solution was dumped upon it but the normal increased metabolic rate did not appear.

As an interpretation of these results it may be suggested that the ratio of potassium to sodium outside the muscle fibres is already so far increased by the previous treatment that the succeeding treatment can have no further effect. This favors the view that *sugar, potassium and spontaneous non-irritability are all due to the same cause,* presumably disturbances in the K/Na ratio. The chief difficulty in this interpretation is the prolonged electropositivity of the muscle under sugar as compared to the electronegativity under potassium and in spontaneous non-irritability. (Foster and Moyle, 1921, found a decreased injury current in spontaneous non-irritability.) Probably the absence of all electrolytes in the sugar accounts for this difference there being in fact an initial negativity with sugar.

The effects of acid and alkali on the oxygen consumption rise due to sugar. Determinations were made of the rise in oxygen consumption caused by mixtures of isotonic sucrose solutions with isotonic solutions of HCl and NaOH in various proportions. The results are shown in figure 4,A and B. In figure 4A is included one curve for the effect of sucrose alone which is

fairly typical but shows one feature which has been somewhat rare in my experience, namely, the secondary increase in the oxygen consumption which comes 6 to 7 hours after the addition of the sugar and which may be due to the rigor which supervenes in time. In comparison to the curve for pure sugar it can be seen from figure 4A that the admixture of HCl progressively decreases the rise in the oxygen consumption while figure 4B shows that the admixture of NaOH causes an initial increase in small concentrations, followed by a decrease in mixtures as strong as 30 parts NaOH to 70 parts of sucrose. The effect of such strong concentrations of HCl and NaOH is doubtless due to interference with the oxidizing mech-

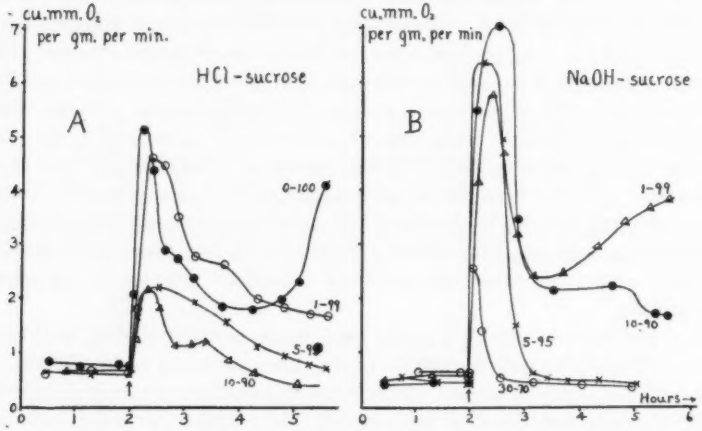

Fig. 4. The effects of various solutions dumped on sartorius muscles at the points marked by arrows. A. Mixtures of 0.1M HCl and 6.5 per cent sucrose. B. Mixtures of 0.1M NaOH with 6.5 per cent sucrose. The effect of sugar alone is in A, marked 0–100. The relative proportions of solutions used are marked on the curves, i.e., 5 cc. NaOH to 95 cc. of sucrose. The following pairs of curves were from match muscles: NaOH 1:99 and 10:90 (92 and 91 mgm.); HCl 1:99 and 5:95 (68 and 76 mgm.); sucrose alone and 30:70 NaOH sucrose (97 and 110 mgm.).

anism proper, the more significant physiological result being that obtained from weak solutions. It may be concluded therefore that *alkali increases while acid decreases the rise in oxygen consumption caused by sugar.*[1]

The increased oxygen consumption caused by sugar is not due to burning of

[1] Alkaline sugar solutions maintain irritability longer than acid solutions. Mixtures of 95 parts of 7 per cent sucrose with 5 parts of 0.1M HCl or NaOH were used for comparison. One pair of ileofibularis and one pair of sartorius muscles were used for comparison. One muscle from each pair was placed in the acid solution and became non-irritable in 9 and 11 minutes respectively. The match muscles in the alkaline solution became non-irritable in 28 and 26 minutes respectively.

the sugar. This follows from the finding that it does not occur with other tissues such as frog skin, kidney, stomach or nerve, or only to a minimal extent. Likewise it fails to occur, as already mentioned, in a muscle which is rendered non-irritable either from failure to soak in Ringer's solution or from soaking in potassium solutions. Such muscles contain all the usual oxidizing enzymes and are in fact consuming oxygen at an abnormally high rate. Treatment with isotonic sucrose nevertheless does not accelerate the consumption of oxygen in such cases. It may be concluded, therefore, that the rise in oxygen consumption due to sugar in a normal muscle is physiological rather than chemical. The sugar solution alone does not of course consume oxygen.

The rise in oxygen consumption due to sugar is inhibited by small amounts of NaCl, KCl and $CaCl_2$. This fact also shows that the rise in oxygen consumption due to sugar is not a purely chemical effect, for the concentration of the sugar is not reduced materially by the addition of such small amounts of electrolytes. Nevertheless its effect on the oxygen consumption is eliminated.

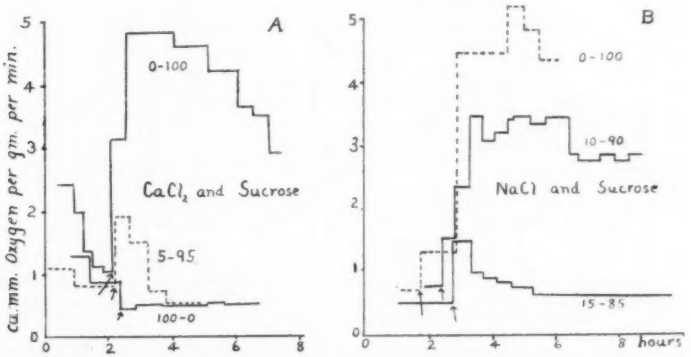

Fig. 5. The effects of mixtures of 6.5 per cent sucrose with various proportions of 0.8 per cent $CaCl_2$ and 0.65 per cent NaCl.

The effects of $CaCl_2$ and NaCl are illustrated in figure 5A. Isotonic solutions of $CaCl_2$ 0.8 per cent or NaCl 0.65 per cent were mixed with sucrose 6.5 per cent in various proportions. In 5 parts of isotonic $CaCl_2$ to 95 parts of sugar there is only a slight temporary rise in rate. A similar experiment with a 10–90 mixture showed a similar but slightly smaller increase. Mixtures of 20 parts $CaCl_2$ to 80 of sucrose showed no rise, while 30–70 mixtures of $CaCl_2$ and sucrose behaved like pure isotonic $CaCl_2$ (100 to 0) as illustrated in figure 5A, and caused an immediate fall to a lower level at which the oxygen consumption remained for rest of the day.

Figure 5B shows similar experiments with mixtures of isotonic NaCl and isotonic sucrose. In mixtures of 15 parts NaCl to 85 of sucrose there is still a small increase in rate but 30–70 mixtures have no effect unless it is an accelerated onset of rigor mortis 5 to 6 hours later. NaCl alone has no effect on the oxygen consumption. I have not attempted to prove that equal amounts of NaCl are necessary for the maintenance of normal irritability as are necessary for the maintenance of normal levels of oxygen consumption but this is at least approximately true.

On the whole, *$CaCl_2$ seems more effective in lowering the oxygen consumption than NaCl.* This is to be expected since $CaCl_2$ alone decreases the oxygen consumption of normal muscles while NaCl does not.

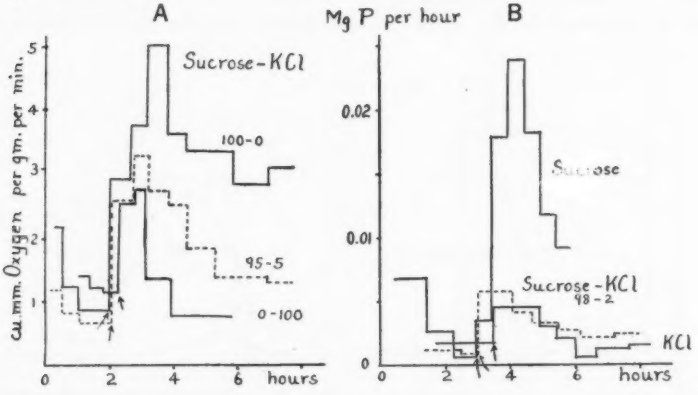

Fig. 6. Effects of mixtures of 6.5 per cent sucrose with 0.9 per cent KCl on A, the oxygen consumption, and B, on the diffusion of inorganic phosphorus from the muscle. Note the similarity between A and B.

Figure 6A shows an experiment with mixtures of isotonic KCl (0.9 per cent) and sucrose. A mixture of 5 parts KCl to 95 parts sucrose has only a slightly larger effect than KCl alone. Ten parts of KCl to 90 of sucrose is indistinguishable from pure KCl. From this experiment the rather striking conclusion may be drawn that the addition of small amounts of KCl to the sugar solutions causes a decrease in the oxygen consumption but an increase in the contracture produced. Either there is a twofold stimulus to the oxygen consumption in sugar solutions, one of which is abolished by KCl, or the development of tension after sugar treatment takes place much more economically in the presence of added KCl.

It may be mentioned that it is not of course possible to make all these experiments on match muscles so that all the results are not quantitatively comparable with each other. This difficulty is not serious, however, be-

cause the percentage differences in the effects of the various solutions are very large.

Phosphoric acid liberation. For comparison with this effect of KCl on the oxygen consumption I have measured the loss of phosphoric acid from the muscle according to the method described by Embden (1921). The results are shown in figure 6B. As Embden showed, the diffusion of PO_4 is reduced to minimal amounts in Ringer's solution but rises to great heights in isotonic sugar solutions. Here again minimal amounts of KCl added to the sucrose (in this case only 2 parts of isotonic KCl to 98 of sucrose) had practically the same effect as pure KCl. This parallelism between the oxygen consumption and the rate of phosphoric acid loss is

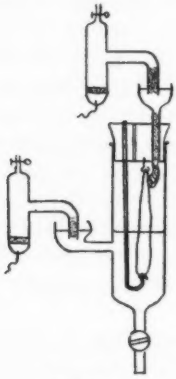

Fig. 7

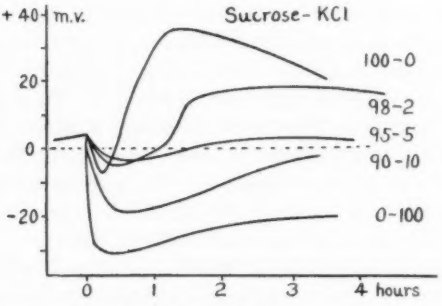

Fig. 8

Fig. 7. Apparatus used in measuring the potential difference caused by immersing the lower end of a sartorius muscle in various solutions.

Fig. 8. Graphs of measurements obtained by the apparatus of figure 7. Effects of various mixtures of isotonic solutions of sucrose and KCl. A positive potential indicates that the immersed end of the muscle was positive in relation to the untreated exposed end of the muscle.

very striking, and suggests that both these factors must be controlled by the same fundamental cause. Embden has suggested an increase in permeability as an explanation. Eggleton (1929) however believes that the increased phosphoric acid excretion may be explained by an increased concentration of this substance inside the muscle fibres.

Any salt added to sugar solution, even in small amounts, becomes at once the dominant salt in the solution. Hence it is not surprising that a solution of 2 parts KCl to 98 of sucrose should behave like pure KCl. It is not so easy to explain why sucrose alone should have so much more effect on O_2 consumption and phosphoric acid liberation than KCl alone.

The changes in potential of the muscle caused by sugar solutions. This

subject was studied by means of the apparatus illustrated in figure 7.[2] One end of the sartorius was tied against a piece of cotton moistened with Ringer's solution which was in contact, through a column of 15 per cent gelatin in Ringer's solution, with a calomel electrode. The lower end of the muscle was tied to a glass hook and dipped into the solution to be investigated, which was in electrical connection through a side arm with the second calomel electrode. The potential difference was measured by a null method in the usual way.

The effect of mixtures of sucrose and KCl on the potential difference is illustrated by the graphs of figure 8. The muscle starts in Ringer's solution and the immersed end is always a little more positive than the exposed (upper end). When pure isotonic KCl is used to replace the Ringer's solution there develops an electronegativity of the immersed end of some 30 millivolts which persists for hours and resembles the change produced by other substances causing contracture of the muscle. Pure sucrose on the other hand causes an initial development of electronegativity of about 10 millivolts which is followed within 30 minutes or less by a prolonged electropositivity of some 30 to 40 millivolts. This agrees with the fact observed by Höber (1905) that the injury current produced by KCl is much increased by previously soaking the muscle in sugar. The positivity phase comes on more rapidly if the sucrose is repeatedly changed so that electrolytes escaping from the muscle are removed. Similar changes also occur with distilled water (Oker-Blom, 1901).

As the concentration of KCl in the sucrose is increased the electropositive effect disappears and only an increased electronegative phase persists. Mixtures of 5 parts of KCl to 95 of sucrose which were sufficient to reduce the curves of phosphoric acid loss and the oxygen consumption to the level reached by KCl alone are also sufficient to abolish completely the electropositive effect of the sucrose. *The greater part therefore of the increased oxygen consumption is associated with an electropositivity of the muscle* and an increased phosphoric acid excretion. The explanation of this positivity is not clear. Oker-Blom (1901) suggested that a similar effect observed in distilled water was a diffusion potential due to the escape of lactic acid from the muscle. The fact that the positivity is decreased or abolished when the sugar contains lactic or hydrochloric acid is qualitative evidence in favor of this theory. The initial negativity might possibly be related to the contracture caused by sugar.

The contraction due to sugar. I have found no mention in the literature of the fact that there is a mechanical change in muscles immersed in isotonic sugar solutions except for some spasmodic twitching mentioned by Overton. Yet this contracture may be important for the explanation of

[2] A somewhat similar apparatus was used by E. Fischer (Pflüger's Arch., cciii, 580. 1924).

the effects observed. The tension develops rather slowly and slowly decreases. It never amounts to more than 2 or 3 grams at the maximum and lasts 15 to 20 minutes in all. Occasionally there is no tension due to sugar but the reason for this failure has never been apparent. The contraction does not depend upon the acidity of the sugar solutions which have been carefully neutralized with NaOH, KOH and phosphate buffers without changing the effect except when the salt concentration becomes appreciable. I have used the purest sugar obtainable (Pfanstiehl) but the result seems to be identical with that observed in ordinary table sugar.

Like other contractures, that due to sugar is accompanied by an increased oxygen consumption, an increased phosphoric acid escape and an increased lactic acid concentration (Hegnauer, 1931). Certain peculiarities may, however, be noted. Thus the contracture may be vanishingly small or even absent without a corresponding decrease in the excess oxygen consumption. Even when the largest actual contractions are obtained, the ratio of the oxygen (cu. mm. per cm. length of muscle) to the tension time produced (gm. sec.) is as large as 0.065 for sugar, as compared to 0.04 for KCl 0.9 per cent, and 0.009 for electrical stimulation (cf. Fenn, 1931). The process of transforming chemical energy into mechanical effect may evidently become extremely inefficient under sugar treatment, or else part of the increased metabolism is associated with an "increased permeability" of the muscle as Embden suggested.

The return of the oxygen consumption to normal levels after treatment with sucrose. In most cases the oxygen rate declines only very gradually after the increase due to sugar but sometimes the decrease is much more rapid. I tried many experiments to find out what factors might account for this occasional prompt return of the oxygen consumption to normal levels.

On the theory that the oxygen intake came down because the lactic acid concentration was diminished, I tried adding small amounts of lactic acid to the sugar solutions after the muscles had been immersed in them for about 2 hours. The result, however, was a prompt and marked *decrease* in the rate of oxygen consumption (rather than an increase) like that illustrated in figure 3A for KCl.

In other experiments, various electrolytes were added to the sugar solutions in which the muscles had been immersed for an hour or more. In every case, the oxygen consumption showed a prompt decrease irrespective of the nature of the electrolyte used. This effect was produced by 0.023 per cent NaCl, 0.01 M and 0.026 M HCl, and by isotonic KCl and $CaCl_2$ solutions. Even electrolytes like HCl and KCl, which cause a rise in oxygen in normal muscles, produce a rapid fall in oxygen consumption after sugar treatment. One electrolyte which might temporarily increase the oxygen consumption after sugar is NaOH for the effect of sugar on the metabolism is slightly increased if it is made alkaline (fig. 4B). The only

substance which I have tried which increased the metabolism of a muscle made non-irritable from sugar is methylene blue and this, presumably, is purely a chemical effect upon the oxidizing system.

From these results it would appear that it is the accumulation of electrolytes diffusing out of the muscle into the solution bathing it which is responsible for the return of the oxygen consumption to normal. The principal electrolyte which diffuses out of a muscle into sugar solutions is potassium phosphate. When this substance was added to the sugar in a concentration of 0.01 M there was in fact a prompt decrease of the oxygen consumption from 3.5 to 0.5 cu. mm. per gram per minute. This experiment seemed to confirm the theory but other experiments showed it to be incomplete. Thus I tried removing the sugar solution from the respirometer at half hour intervals and replacing it by more of the same solution, thinking by this means to dilute the electrolytes as fast as they diffused out and so to keep the oxygen consumption at a high level. The reverse took place, however, for the oxygen consumption came lower the more frequently the sugar solution was changed. This result, which was confirmed many times, suggested that some substance such as Meyerhof's coferment was being removed from the muscle by all of these solutions which, when left to accumulate, would keep the oxygen consumption high. I have been unsuccessful, however, in demonstrating the presence of such a substance,[3] and I must therefore leave the question to future investigation for a final answer.

Since further experimental study of the relation between muscle metabolism and its physico-chemical environment is still in progress, discussion of the results herein presented is best reserved until all the facts known can be considered together.

I am greatly indebted for much technical help in these experiments to my assistant, Mr. W. B. Latchford, and for corroborative experiments to my associates, Mr. B. Marsh and Mr. A. H. Hegnauer, who are continuing the investigation of certain aspects of this subject.

SUMMARY

1. The immersion of muscles in isotonic sucrose solutions is accompanied by a small contraction of the muscle, an enormous increase in the oxygen

[3] Two match muscles were put into 6.5 per cent sucrose in two respirometers. In one of these the sugar solution was changed every half-hour and the oxygen consumption came down from 3.2 cu. mm. to 1.1 cu. mm. per gram per minute. At the same time the control muscle had a rate of 3.1 cu. mm. The solution in which this muscle was bathed presumably contained this hypothetical substance in high concentration. The muscle with the low rate was therefore transferred to this solution in the expectation that its rate of oxygen consumption would increase. Instead its rate fell still further from 1.1 cu. mm. to 0.9 cu. mm. per gram per minute.

consumption, an increased loss of phosphoric acid, an increased lactic acid concentration, a loss of irritability, and an electrical change in the muscle which is temporarily negative but predominantly positive.

2. Mixtures of small amounts of isotonic solutions of NaCl, $CaCl_2$, and KCl with isotonic sugar solutions behave like isotonic solutions of these salts without sugar.

3. The immediate increase in the oxygen consumption caused by sugar is increased by NaOH and decreased by HCl.

4. As a rule the oxygen consumption remains high almost indefinitely in sugar solutions unless these solutions are constantly renewed or unless they receive additions of electrolyte. Apparently any electrolyte so added brings the oxygen consumption back to normal levels.

5. In spontaneous non-irritability no rise in oxygen consumption is caused by sugar, potassium or other agents causing contracture.

6. Previous treatment with potassium prevents the rise in oxygen consumption due to sugar.

7. Previous treatment with sugar prevents the contracture and the rise in oxygen consumption due to potassium.

8. It is suggested that sugar non-irritability, potassium non-irritability and spontaneous non-irritability are all due to the same fundamental cause, which is probably a critical shift in the electrolyte balance across the cell membrane.

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THE EFFECTS OF ADRENALIN, EPHEDRINE, AND INSULIN ON BLOOD FAT[1]

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The antagonistic effects of adrenalin and insulin are manifested on blood sugar, adrenalin relieving insulin hypoglycemia and insulin diminishing adrenalin hyperglycemia. Similar antagonistic actions have not been demonstrated for blood fat, although it is known that insulin may lower the level of plasma fat when it is raised as in diabetes (Campbell and Macleod, 1924), or alimentary lipemia (Rony and Ching, 1930), and even when it is not raised under post-absorptive conditions (Christomanos, 1929). However, during hypoglycemia there may be an increased concentration of fat (Raper and Smith, 1925). The effects of adrenalin on blood fat are variable, probably depending on the experimental conditions. Alpern and Callazo (1923), Raab (1926), and Alpern and Tutkewitsch (1929), have shown that adrenalin may cause a decrease in blood fat, while Bornstein and Müller (1921) did not find definite changes. Fleisch (1926) observed no characteristic results in the first few hours after the injection, but within 24 and sometimes 48 hours, obtained a great increase in fat. In the present experiments the effects on plasma fat, blood sugar, and lactic acid of adrenalin and insulin given separately or together were studied on unanesthetized post-absorptive dogs.

Although ephedrine is similar to adrenalin chemically and in some of its physiological actions, for example, in raising the level of blood sugar (Chen and Schmidt, 1930), it nevertheless differs from adrenalin in respect to some other reactions (DeEds, 1927; Tainter, 1929; Curtis, 1929; Finkleman, 1930). It therefore becomes of interest to compare the effects of ephedrine with those of adrenalin. Accordingly, determinations of fat, sugar, and lactic acid were made, after the administration of ephedrine with and without insulin.

METHOD. Adrenalin chloride (0.1 per cent solution) from Parke, Davis & Company[2] was given subcutaneously to unanesthetized dogs weighing

[1] Preliminary reports appeared in the Proc. Soc. Exper. Biol. and Med., 1930, xxvii, 814, and ibid., 1930, xxviii, 333.

[2] We wish to express our thanks to Parke, Davis & Company for the adrenalin chloride, and Eli Lilly & Company for the insulin.

from 7 to 12 kilos, either in single doses ($\frac{1}{16}$ cc. to 2 cc.) or in doses ($\frac{1}{8}$ cc. to 1 cc.) repeated every $\frac{1}{4}$ hour from 2 to 6 hours. Ephedrine sulphate from Eli Lilly and Company[2] (3 per cent solution) was injected in divided doses every $\frac{1}{4}$ hour from 2 to 5 hours into 12 amytalized dogs varying in weight from 4 to 18 kilos. The dosage of insulin was 1 or 2 units per kilo. Blood samples drawn from the femoral artery were analyzed for glucose by the technique of Hagedorn and Jensen (1923) and fat according to our modification (Himwich, Friedman and Spiers, 1931) of the method of Stewart and White (1925), differences of 40 mgm. being considered siginficant. Total solids of the plasma were also estimated, thus permitting results obtained for fat to be corrected for changes in concentration. Lactic acid was determined by the procedure of Friedemann, Cotonio, and Shaffer (1917).

Results. Eight experiments of which nos. 1 and 2 are examples were done on dogs which had received insulin and in each case the fat diminished. The characteristic effect on blood sugar may also be noted. Experiments 3 and 4 are typical of the action of adrenalin, since that substance caused a greater concentration of the fat content of the plasma in each of 15 experiments. The plasma fat in experiment 3 decreased at 6 hours despite continued injections of adrenalin. The diminished effects of the later doses of adrenalin are also apparent in regard to blood sugar and lactic acid. In experiment 4 fat rose in 4 hours, although the injections of adrenalin were discontinued at 2 hours.

In a series of 12 experiments both insulin and adrenalin were given and the antagonistic effects were apparent in every instance. In some observations like that of the fifth experiment in table 1 adrenalin overcame the effects of insulin. In the sixth experiment the action of adrenalin on fat was prepotent at 2 hours, although glucose fell distinctly at the same time. However, by the sixth hour the insulin effect became apparent on fat too. It will be observed that the lactic acid of the blood continued to rise despite the injection of insulin. Still another relationship may be seen in the seventh experiment in which the action of insulin on fat and glucose was marked by the second hour, although in the fourth there was a beginning of adrenalin preponderance on the concentration of fat, a phenomenon which was even more marked in the case of glucose. In another series of 7 experiments the effect of a single injection of adrenalin was observed. Beginning with a dose as large as 2 cc. and finally decreasing to $\frac{1}{8}$ cc. in a 7 kilo dog, the invariable result was a rise in blood fat. However, when $\frac{1}{10}$ cc. was injected in a dog weighing 10.7 kilos (expt. 8) the concentration of the plasma fat decreased, even though adrenalin had its usual effect on blood sugar. A similar result was noted after $\frac{1}{16}$ cc. adrenalin was given to a dog of 8.7 kilos.

In table 2 are presented the results of one experiment with ephedrine and two others with ephedrine and insulin. In 10 experiments with ephedrine

TABLE 1

Effects of adrenalin and insulin on plasma fat, blood sugar and lactic acid

EXPERIMENT NUMBER	TIME	PLASMA FAT	BLOOD SUGAR	BLOOD LACTIC ACID	WEIGHT	DOSAGE OF EXTRACTS
		mgm. per cent	*mgm. per cent*	*mgm. per cent*		
1	Pre-injection	507	140		7.0	Insulin
	2 hours	436	96			2 units per kilo
	4 hours	354	75			
2	Pre-injection	830	104		8.6	Insulin
	2 hours	533	61			2 units per kilo
	4 hours	663	45			
3	Pre-injection	708	110	13	10.0	Adrenalin, $\frac{1}{8}$ cc. every $\frac{1}{4}$ hr.
	2 hours	762	153	16		for 6 hrs., or 0.05 mgm.
	4 hours	1,156	264	25		per kilo per hour
	6 hours	801	229	18		
4	Pre-injection	537	120		10.0	Adrenalin, $\frac{1}{2}$ cc. every $\frac{1}{4}$ hr.
	2 hours	840	177			for 2 hrs., or 0.2 mgm.
	4 hours	893	213			per kilo per hour
	6 hours	794	199			
5	Pre-injection	327	110	15	8.5	Insulin, 1 unit per kilo.
	2 hours	457	117	41		Adrenalin $\frac{1}{2}$ cc. every $\frac{1}{4}$ hr.
	4 hours	511	199	39		for 2 hrs., or 0.23 mgm. per kilo per hour
6	Pre-injection	679	100	16	7.0	Insulin, 2 units per kilo.
	2 hours	719	86	31		Adrenalin, $\frac{1}{2}$ cc. every $\frac{1}{4}$ hr.
	4 hours	644	83	39		for 2 hrs. 0.29 mgm. per kilo per hour
7	Pre-injection	484	111	17	10.0	Insulin 1 unit per kilo.
	2 hours	307	103	39		Adrenalin, $\frac{1}{4}$ cc. every $\frac{1}{4}$ hr.
	4 hours	370	139	20		for 4 hrs. 0.1 mgm. per kilo per hour
8	Pre-injection	735	101		10.7	$\frac{1}{10}$ cc. adrenalin
	2 hours	689	124			
	4 hours	616	110			
	6 hours	714	110			

of which the first of table 2 is an example, there was a consistent rise of the blood sugar level. Increases in fat occurred in only 7 experiments, while

the concentration of the lactic acid of the blood was augmented in but 5 observations. In some of the experiments it was impossible to correct the fat results for changes in concentration of the plasma, since the determination of total solids was unsatisfactory because of hemolysis, an effect of ephedrine. It will be observed in the second experiment of table 2 that the insulin action overcame that of ephedrine. In the third experiment injections of ephedrine were started 2 hours before that of insulin, but by the fourth hour characteristic insulin results were observed.

DISCUSSION. Injections of adrenalin not only cause an increased concentration of blood fat, but also of the lactates and acetone bodies of the

TABLE 2

Effects of ephedrine and insulin on plasma fat and blood sugar and lactic acid

EXPERIMENT NUMBER	TIME	PLASMA FAT	BLOOD SUGAR	BLOOD LACTIC ACID	WEIGHT	DOSAGE OF EXTRACTS
		mgm. per cent	*mgm. per cent*	*mgm. per cent*		
1	Pre-injection	705	110	44	8.0	Ephedrine, ½ cc. every ¼ hr. for 4 hrs. 7.5 mgm. per kilo per hour
	2 hours	1,120	183	59		
	4 hours	807	134	60		
	6 hours	696	125	71		
	8 hours	697	125	48		
2	Pre-injection	893	94	23	12.0	Insulin, 1 unit per kilo. Ephedrine, ½ cc. every ¼ hr. for 4 hrs. or 5 mgm. per kilo per hour
	2 hours	570	72	25		
	4 hours	622	51	33		
3	Pre-injection	579	93	15	9.0	Ephedrine, ¼ cc. every ¼ hr. for 4 hrs. or 3.3 mgm. per kilo per hour. Insulin, 1 unit per kilo injected at 2 hrs.
	2 hours	694	121	17		
	4 hours	493	44	23		
	6 hours		31	19		

blood. Since the method employed for the estimation of fat (Himwich, Friedman and Spiers, 1931) probably determines fatty acids only in ester combinations, neither lactates nor acetone bodies were included in our results.

Most previous observers have used other methods for the determination of fat, but the differences between the results they obtained and the present ones are not necessarily to be imputed to the method employed, but rather to the magnitude of the dosage. Alpern and Collazo (1923), Raab (1926), and Alpern and Tutkewitsch (1929), employed small doses of adrenalin varying from $\frac{1}{10}$ milligram to 1 milligram and thus produced a decrease of fat. Fleisch's (1926) dose of 3 to 6 mgm. for a rabbit is relatively

enormous and produced no change in blood fat in the hours immediately after the injection, although a great increase usually occurred within 24 hours. On the other hand, more moderate amounts of adrenalin were employed in the present experiments whether injected in single or repeated doses, and invariably produced a gradual rise in blood fat.

The changes of fat after injections of adrenalin and insulin may be attributed to their influence on the character of the foodstuffs oxidized. Wilenko (1912) observed no rise in respiratory quotient after the administration of glucose and adrenalin. Cori and Cori (1928) have demonstrated that in unanesthetized animals the rise in metabolic rate caused by injections of adrenalin (Boothby and Sandiford, 1923) is due in largest measure to the increased oxidation of fat. Colwell and Bright (1930) found that under continued injections of adrenalin the respiratory quotient of cats under amytal anesthesia approaches the diabetic level. Adrenalin apparently diminishes the oxidation of carbohydrates making for an increased combustion of fat. The first effect as seen with small doses of adrenalin is a diminution of blood fat. Probably all the tissues are removing increased amounts of fat from the blood in order to supply the energy necessary for the maintenance of the life processes. With moderate doses of adrenalin the increased removal of blood fat is masked by the mobilization of the fat depots. Since blood fat increases the tissues are probably not removing fat from the blood as rapidly as it is being liberated, although an increased fat content of liver (Junkersdorf and Török, 1926; Störring, 1928) and of kidney (Junkersdorf and Török, 1926) has been noted after injections of adrenalin in dogs. Since insulin accelerates the combustion of carbohydrates and renders a supply of fat less necessary, the decrease in blood fat produced by insulin must have occurred despite diminished utilization by the tissues. The phenomenon may be explained by an increased storage of fat in the depots. Raper and Smith (1925) have shown that insulin decreases the fat content of the liver, while augmenting that of muscle. Apparently fat becomes less important for the active metabolic processes.

Ephedrine exhibits the physiological actions of adrenalin but to a lesser degree, since the results of ephedrine injections on fat and lactic acid were not so consistent as those of adrenalin. Moreover, effective doses of ephedrine were often toxic. As in the case of adrenalin, so after injections of ephedrine, insulin was capable of successfully antagonizing the rise of fat and glucose.

There was no apparent inhibition of insulin on the accumulation of the blood lactic acid caused by adrenalin or ephedrine injections. These results agree with those of Cori and Cori (1929).

CONCLUSION

Dogs have been injected subcutaneously with insulin or adrenalin or both, and fat, total solids of plasma, blood sugar, and lactic acid were estimated. It was found that insulin lowered the post-absorptive level of the plasma fat, while adrenalin in moderate doses, invariably raised the level. As might be expected when both of these endocrine products were injected into the same animal an antagonism was exhibited, adrenalin or insulin being predominant according to the magnitude of their respective dosages. Adrenalin in small amounts however caused a decreased concentration of blood fat.

Ephedrine injections always produced a rise in the level of blood sugar, but the effects on plasma fat and blood lactic acid were not so consistent. Insulin successfully antagonized the effects of ephedrine on fat and sugar.

Under the present experimental conditions it was not possible to demonstrate a diminution in the concentration of blood lactic acid as an action of insulin.

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BILE PIGMENT FORMATION IN THE LIVER FROM HEMOGLOBIN

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Most investigators are today of the belief that hemoglobin is the chief, if not the only source of bile pigment and for the following reasons: *a.* Hematoidin is identical with bilirubin. Since this idea was first expressed by Virchow (1) in 1847, Robin Hoppe-Seyler, Holm, Preyer (2), van den Bergh and Snapper (3), Hooper and Whipple (4), Fischer and Reindel (5), Rich and Bumstead (6) and others have brought experimental evidence in its support. *b.* When hemoglobin or its derivatives are injected into the blood, the bile pigment of the bile is increased. Tarchanoff (2) first reported this fact in 1847, since when Brugsch and Yoshimoto (7), Brugsch and Kawashima (8), Barratt and Yorke (9), Whipple and Hooper (10) and others have verified it. *c.* The formation of bile pigment from hemoglobin in tissue cultures as studied by Rich (11) and by Parisot (12). Perfusion of the liver by a method which I have previously described (13) offers a simple means of studying the formation and elimination of bile pigment. Schmulewitsch, Schiff, Pflüger, Rohrig (2) and others have used this method, but mainly on various warm-blooded animals. In my own experiments I have used the bull frog (Rana catesbiana) as a more hardy animal for experimental purposes.

METHOD AND RESULTS. My frogs weighed 400 to 600 grams; their livers 7 to 20 grams. Ether anesthesia was used. Ringer solution at constant pressure and nearly constant oxygen content was perfused simultaneously into the hepatic artery and portal vein, exit being permitted from the hepatic vein. To eliminate error I used the perfusion apparatus previously described (13), maintaining the pressure at 260 mm. of water in the hepatic artery and at 60 mm. in the portal vein. A glass cannula about 1 mm. in diameter was fastened into the cystic duct, from which flowed a water-white liquid at the rate of 0.005 to 0.01 cc. per 10 minutes. This was tested 1, as to its color; 2, by Gmelin's test, and 3, spectroscopically in *a*, the visible and *b*, the ultraviolet portions of the spectrum. The cystic bile of the bull frog may be bluish green, brown or yellow. The yellow bile pigment gives no reaction to Gmelin's test but the brown

and the bluish green pigments yield a pale rose color. The pigments of cystic bile show no absorption bands in the visible spectrum but a clearly defined one in the ultraviolet (13).

Two kinds of hemoglobin were used in my experiments, a crude hemoglobin prepared from horse red blood corpuscles (washed three times with 1.2 per cent sodium chloride and dried on a glass plate in the air at 30°C.), and crystallized horse hemoglobin prepared by electro-dialysis (14). The two preparations at 0.2 per cent or 0.1 per cent concentration respectively in Ringer solution gave results nearly identical with a 2.0 per cent defibrinated toad's blood in Ringer solution (13). The protocols which follow have been selected from numerous experiments, all giving uniform results:

Experiment 1. May 2, 1929; room temperature 25°C.; temperature of perfusion fluids, 22°C. A female bull frog weighing 510 grams and with a liver weighing 11 grams is perfused as described above, with a Ringer solution followed by one containing 0.1 per cent crystallized horse hemoglobin.

The liver is first perfused with plain Ringer solution until the cannula in the cystic duct yields a water-clear fluid. The Ringer solution flowing into the hepatic artery is now replaced by one containing hemoglobin. In twenty minutes a deep bluish green fluid is obtained which the previously described tests show to be identical with the normal bluish green bile pigment of the gall bladder. This experiment proves that hemoglobin is changed into bile pigment in the liver of the bull frog.

The question of whether bile pigment is produced via the hepatic artery or the portal vein is discussed by Legg (2). He holds that those investigations which involve ligation of the portal vein or the hepatic artery are inconclusive. The following experiment applies to this point.

Experiment 2. April 25, 1929; room temperature, 25.5°C.; temperature of perfusion fluids 22°C. A female bull frog weighing 476 grams and with a liver weight of 8 grams is first perfused through hepatic artery and portal vein with Ringer solution until the fluid flowing from the cystic duct is colorless. For the plain Ringer solution infused through the portal vein is now substituted one containing 0.2 per cent crude hemoglobin. Even after one and a half hours no pigment appears in the cystic duct. But when a similar perfusion is carried out via the hepatic artery, with plain Ringer solution flowing into the portal vein, a deep bluish green pigment appears in twenty minutes. Examination shows this pigment to be identical with the normal bluish green bile pigment of the gall bladder.

This experiment indicates that the hepatic artery but not the portal vein plays a direct part in the formation of bile pigment in the liver under these conditions.

I have previously reported experiments which show that the elimination of dyes from the liver is inhibited by hydrogen cyanide (13). I repeated this experiment on the elimination of bile pigment from the liver after hemoglobin perfusion.

Experiment 3. June 21, 1929; room temperature, 30°C.; temperature of perfusion fluids 26°C. A male bull frog weighing 590 grams and with a liver weight of 13 grams receives an initial liver perfusion with plain Ringer solution. When the fluid flowing from the cystic duct has cleared, the plain Ringer solution entering the portal vein is replaced by one containing hydrogen cyanide (made by neutralizing a 1/600 mol. KCN. solution in Ringer with HCl, using neutral red as an indicator) while the fluid entering the hepatic artery is replaced by one containing 0.2 per cent crude horse hemoglobin. Though perfusion is continued one and a half hours, no pigment appears in the fluid coming from the cystic duct. The Ringer solution containing cyanide is now replaced by plain Ringer. After an hour, a bluish green pigment is eliminated which gradually deepens in color. The same fluids are again used, but with the arrangement reversed: HCN flows with hemoglobin through the hepatic artery and the plain Ringer solution through the portal vein. The results are the same.

This failure of bile pigment to appear shows that either the production or elimination of bile pigment from the liver is suppressed through poisoning with hydrogen cyanide, but that the elimination of water is not affected.

CONCLUSIONS

1. The bull frog is a favorable experimental animal upon which to study the formation and elimination of bile pigment. It is hardy and perfusion of the liver is easily carried out.

2. Suitable perfusion with dilute hemoglobin solution is followed by bile pigment formation and elimination proving that hemoglobin is converted into bile pigment in the liver.

3. Perfusion of hemoglobin through the hepatic artery leads to bile pigment formation but not perfusion through the portal vein.

4. The appearance of bile pigment in the cystic duct after perfusion with hemoglobin is inhibited by hydrogen cyanide.

I should like to express my heartfelt thanks to Prof. S. Oinuma and Prof. M. H. Fischer, both of whom have been very helpful with advice during the course of the work.

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THE INFLUENCE OF MENTAL ACTIVITY ON THE HEIGHT OF THE KNEE-JERK

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The knee-jerk is usually considered an index of muscle tonus and, therefore, easily influenced by many external and internal factors. The increase in the knee-jerk caused by a problem in addition (Tuttle 1924 a), and the slight contraction of a muscle produced by imagination (Jacobson, 1930) are good illustrations of changes in muscle tonus brought about by mental activity. The present report shows the effect of imagination on the height of the knee-jerk.

Experimental method and results. The knee-jerk apparatus used was, as previously described (Emery, 1929), a modification of Tuttle's (1924) machine. The subjects were all men students who were in their third year of college work. Each subject was given a preliminary trial period and on the following day the experimental data were obtained. These men were, therefore, familiar with the knee-jerk but were not well trained subjects; neither were they told what results were expected.

The experiments were performed in a quiet room and consisted of A, first normal period; B, a period during imagining kicking a foot ball; C, second normal period, and D, a period during imagining kicking back or spurring a horse. The interval of rest between periods was usually less than one minute. The usual procedure was to give a signal, by tapping a rod on metal, about one second before the hammer hit the knee. In both normal periods (A and C) the subject remained passive when he heard the tap, but in periods B and D he imagined performing movements of the leg as previously instructed. Forty subjects were studied and ten or more knee-jerks were obtained for each of the four periods. These data have been analyzed and tabulated in table 1. In order to reduce the tables the individual knee-jerks are not given; however, all the data are calculated from them. It was found, as shown in table 1, that only three of the subjects showed a decrease in height of the knee-jerk during the period they were imagining kicking the foot forward; and also only three subjects responded with an increase in the knee-jerk when they were imagining kicking backward. In other words, a total of eighty cases compared to the normal showed that only six were in the direction opposite of expecta-

TABLE 1

The average height of the normal knee-jerk in millimeters is shown under A, and a second normal under C. Period B shows the increase in millimeters above the normal period A, and period D shows the decrease in millimeters below the normal period C. These changes were brought about by imagining movements of the foot as indicated. The data for each subject are calculated from the average height of ten knee-jerks for each of the periods.

SUBJECT NUMBER	A NORMAL	B IMAGINING KICKING FORWARD	MEAN DIF. / P.E. OF DIF.	C NORMAL	D IMAGINING KICKING BACKWARD	MEAN DIF. / P.E OF DIF.
1	13.9	+6.0	6.0	14.3	−7.5	6.0
2	14.1	+2.1	1.6	16.8	+2.2	2.2
3	26.4	+4.5	4.3	23.4	−9.6	9.0
4	16.1	+0.2	0.1	14.9	−2.7	1.7
5	14.9	−0.3	0.4	7.0	−3.5	3.1
6	23.1	+2.3	3.0	18.3	−11.6	8.8
7	23.2	+8.1	8.9	19.6	−10.3	9.6
8	12.3	+1.7	1.4	12.6	−0.5	0.4
9	11.5	+5.0	7.0	10.7	+0.8	0.8
10	29.5	+14.3	14.0	28.2	−7.6	9.0
11	11.7	+4.5	3.6	15.6	−4.6	3.8
12	17.5	+4.2	3.1	15.5	−5.6	6.6
13	16.8	+5.6	4.0	13.2	−13.0	20.6
14	13.1	+2.2	2.6	11.9	−7.9	7.8
15	26.2	+16.8	17.1	27.5	−2.9	1.7
16	19.0	+0.5	0.5	14.4	−0.9	0.9
17	10.2	+3.4	4.0	7.6	−3.6	5.8
18	19.6	−0.3	0.3	17.2	−8.9	5.1
19	14.1	+5.5	5.0	15.4	−7.1	12.0
20	26.0	+9.0	17.5	23.8	−1.3	2.5
21	13.9	+3.5	2.3	16.1	−10.6	5.6
22	21.4	+5.2	13.7	23.8	−1.3	2.4
23	25.4	+11.1	16.2	25.0	−13.7	8.5
24	17.9	−0.7	0.3	15.8	−8.8	6.0
25	19.3	+4.8	7.8	18.8	−5.9	4.6
26	17.5	+1.0	1.2	15.3	−2.0	1.3
27	15.1	+8.8	7.4	13.8	−3.7	2.6
28	6.1	+10.7	13.0	6.9	−5.5	5.5
29	18.5	+2.8	5.1	19.4	−3.1	5.9
30	19.3	+13.1	8.2	19.3	−8.3	4.3
31	12.9	+9.9	6.5	16.7	−14.2	13.4
32	20.5	+9.5	11.8	18.8	−5.7	6.6
33	18.9	+1.4	1.3	17.4	−3.2	4.0
34	22.7	+8.1	8.9	23.3	−2.5	3.5
35	10.2	+10.1	8.5	11.7	−5.3	2.8

TABLE 1—*Concluded*

SUBJECT NUMBER	A NORMAL	B IMAGINING KICKING FORWARD	MEAN DIF. / P.E. OF DIF.	C NORMAL	D IMAGINING KICKING BACKWARD	MEAN DIF. / P.E. OF DIF.
36	7.0	+11.1	6.7	10.0	−1.9	1.0
37	19.2	+2.8	3.8	20.5	−7.9	10.4
38	11.3	+5.5	3.8	9.6	−7.1	3.8
39	16.6	+7.9	4.3	12.6	+2.1	1.2
40	21.9	+5.6	5.8	24.6	−7.5	9.7
Average..	17.4	+5.7	6.0	16.7	−5.6	5.5

tion. A further analysis of the data shows the difference between the normal and the experimental periods divided by the probable error of the difference was three or more in fifty-seven out of the eighty possible chances,

Fig. 1. Knee-jerk record of subject 30 showing *A*, a normal period; *B*, subject imagining kicking a foot ball; *C*, a second normal period; and *D*, subject imagining kicking backward.

i.e., the results obtained were significant in 71 per cent of the cases. Since the knee-jerk is always variable in height the positive results obtained, with these untrained subjects, are, therefore, very gratifying.

When the forty subjects are considered as a group the mean of the first normal period is 17.4 millimeters (table 1) and the mean of the second normal period is 16.7; the difference is 0.7 millimeter, a figure that is not significant.

One of the most striking records obtained is shown in figure 1. In this case the normals are identical in height (see subject 30, table 1) the mean of each is 19.3 millimeters, the knee-jerk was increased, during imagining kicking a foot ball, to a mean height of 32.4 millimeters or an increase of 68 per cent. This compares to a mean of 11.0 millimeters during imagining kicking back or a decrease from the normal of 43 per cent. The fact that in this subject the normal periods were of the same average height is incidental; however, this record does clearly show the changes produced in the height of the knee-jerk by imagination.

DISCUSSION. The results show that imagination may increase or decrease the tonus of a muscle or group of muscles. Imagining kicking the foot forward seems to produce a slight increase in tension of the quadriceps muscle which is probably produced by nerve impulses to the muscle as Jacobson (1930) has shown for other muscle groups. Therefore, the increase in the knee-jerk when the subject imagines kicking a foot ball is just what would happen if the muscles involved in the response are already slightly contracting and in a high degree of tonus. Imagining kicking backward or bending the knee may be thought of as producing an increase of tonus in the hamstring group of muscles with an inhibition of the quadriceps group. The knee-jerk if elicited when the quadriceps group is in decreased tonus, perhaps due to reciprocal inhibition in this case, would be less than normal. This idea, suggested as a possible explanation of the results obtained, is in agreement with Sherrington's (1893 and 1905) finding that stretching the hamstring group of muscles diminished the knee-jerk.

SUMMARY

1. The knee-jerk has been obtained during a normal period and during a period when the subject imagined kicking the foot forward; also the knee-jerk was taken during a second normal period and during a period when the subject imagined kicking the foot backward.

2. The data from forty subjects have been analyzed and show the height of the knee-jerk to be increased when the subject imagined kicking the foot forward. There was no significant difference between the two normal periods. The knee-jerk was decreased when the subject imagined kicking the foot backward.

3. These results are probably due to changes in tonus of the muscles involved in the response.

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TOXICITY OF MUSCLE EXTRACTS AFTER ARTERIOTOMY, ASPHYXIATION, INJURIES TO THE BRAIN AND ELECTROCUTION

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In a previous paper (1), one of the authors has described a study on the comparative toxicity of blood after arteriotomy, asphyxiation and injuries to the brain, as determined by phytopharmacological methods. It was shown that blood drawn from animals after injuries to the head, and also after asphyxiation, is much more toxic for living seedlings of *Lupinus albus* grown in plant-physiological solutions than freshly drawn blood after arteriotomy from the same animals. In view of the great sensitiveness of living seedlings for toxins and drugs derived from animals, it was deemed worth while to investigate in a similar manner the relative toxicity of muscle extracts obtained from various animals after different methods of slaughter. The results of such an investigation were very clear-cut and instructive and are reported in this place.

MATERIAL STUDIED. A larger number of animals than those studied in connection with the toxicity of blood and belonging to different genera and species were employed in the present investigation. The following eighteen different kinds of animals were utilized for the purpose: the frog (*Rana clamata*), goldfish (*Carassius aureus*), the carp, the turtle, the domestic hen and the domestic duck, the pigeon, the opossum (*Didelphys virginiana*), the white mouse, the white rat, the guinea pig, the rabbit, kittens, cats, dogs, sheep, calves and oxen.

METHODS OF SLAUGHTER. In the case of frogs, muscle extracts were prepared from the gastrocnemii after decapitation or cutting off the head behind the eyes, on the one hand, and after decerebration or pithing of the brain and spinal cord, on the other. The muscle extracts were prepared about an hour after killing the frogs. In case of goldfish and carp, comparison was made between muscle obtained after decapitation and that taken after decerebration or pithing of the brain. In case of the turtle, comparison was made between muscle extracts obtained after decapitation and after decerebration. In the case of pigeons, comparison was made of muscle extracts obtained after complete exsanguination, on the one hand,

and after breaking the neck of the bird without severing the vessels, on the other. In case of the hen and duck, comparison was made after complete exsanguination, on the one hand, and after decerebration or destruction of the brain with a sharp instrument, on the other. A comparative study of muscle extracts from a male opossum was made in the following way. The animal was anesthetized with ether and several grams of muscle tissue were dissected from one leg, care being taken to clamp and ligate the blood vessels. The animal was then decerebrated through the orbit under ether with a sharp-pointed steel probe and after death muscle tissue was obtained from the opposite leg of the animal; and the extracts in the two cases were compared. Muscle extracts from white mice were obtained after decapitating and bleeding them to complete exsanguination, on the one hand, and after crushing the brain, on the other. In a similar way, comparison was made of muscle extracts obtained from white rats after cutting the throat, on the one hand, and after a blow on the head, on the other. In case of guinea pigs, three kinds of muscle extracts were studied. Some were obtained after arteriotomy and bleeding from severed vessels of the throat. Other extracts were obtained from guinea pigs after ether asphyxiation; and still other specimens were prepared after killing the animals by a blow on the head. In case of rabbits, comparison was made of muscle extracts obtained after arteriotomy and bleeding of the animals, after decapitation, and after electrocution.

The effect of electrocution on the toxicity of muscle extracts was studied on rabbits and cats. The animals were killed with an ordinary alternating current from a lamp socket. One electrode was inserted under the skin of the head and the other over the heart or, in some cases, under the skin of one leg. In all electrocutions the animals were kept under anesthesia throughout the experiment; and the current was allowed to pass through the body for about five minutes. In case of cats, muscle extracts were made after arteriotomy and complete exsanguination, after asphyxiation with ether without exsanguination, after decerebration and after electrocution. In case of dogs, specimens of muscle were taken after arteriotomy and exsanguination, after asphyxiation without exsanguination, and after decerebration. In case of sheep, calves and oxen, slaughtering was not performed by the authors but specimens of fresh muscle were obtained from the abattoir. For comparative purposes the muscles were cut off from the same portions of the body. In each case one specimen was obtained after slaughter of two animals, one of which was killed according to the Hebrew ritual of Šeḥiṭa, that is, by complete severing of the vessels of the throat with a sharp knife and exsanguination of the trussed-up carcass, while the other was killed by a blow on the head.

Method of study. An examination of the various muscle extracts was made in a uniform way. In each case a 2 per cent extract of muscle

tissue was made in a mixture of equal parts of distilled water and Shive solution. The muscle tissue was carefully weighed and cut up finely. It was then ground or macerated thoroughly in a glass mortar for five minutes and filtered. The residue was macerated a second time and filtered again; and the two filtrates were combined. Carefully germinated, healthy seedlings of *Lupinus albus*, with roots measuring from 25 to 40 mm., were then selected and measured. These were placed in upright test tubes of hard glass containing normal control solution, on the one hand, and various muscle extracts, on the other. The complete outfit was placed in the dark at a temperature of 22°C. for from 20 to 24 hours. On the following day the elongation or increment in the length of the roots was measured and the ratio of growth, as compared with that of the normal controls, was expressed as the *phytotoxic index*.

RESULTS AND DISCUSSION. The results obtained are exhibited clearly in the subjoined table. It is interesting to note that the difference in the toxicity of muscle extracts obtained after different conditions of death was noted in all the species of animals studied. It will be noted that in every case the toxicity of the muscle extracts obtained after arteriotomy was less than that of the muscle extracts prepared after decerebration. It will be noted again, in case of frogs, fish and turtles, that there was a marked difference between extracts obtained after decapitation or ablation of the brain and those prepared after decerebration of the brain tissue *in situ*. In those animals in which studies were made of muscle tissues obtained after arteriotomy and after asphyxiation without bleeding, the extract made after asphyxiation was more toxic than that obtained after arteriotomy. This was to be expected in view of the findings already published in connection with the toxicity of blood sera after arteriotomy and asphyxiation.

The causes for the differences noted are a matter for interesting speculation but a number of experimental facts undoubtedly help to explain the above findings. The greater toxicity of some of the muscle extracts examined cannot be ascribed to the effects of ether because when that anesthetic was used all the specimens were obtained under the same conditions of anesthesia and, furthermore, in preparing the extracts practically all the ether was volatilized. Part of the difference in toxicity between exsanguinated and non-exsanguinated muscle must be due to the presence of blood in the latter because the authors have found that normal blood itself, as studied on living seedlings, exhibits an appreciable toxicity for plant protoplasm (2). The difference in toxicity between the muscle extracts from decerebrated and non-decerebrated animals must be attributed to the setting free in the circulation of toxic bodies either from the brain itself or perhaps indirectly, as the result of such injury, from other organs. Positive evidence supporting the view that toxic substances

TABLE 1

ANIMAL	METHOD OF SLAUGHTER	PHYTOTOXIC INDEX
		per cent
Frog, *Rana clamata*	Decapitation	56
	Decerebration	41
Goldfish, *Carassius aureus*	Decapitation	71
	Decerebration	56
Carp, fish	Decapitation	56
	Decerebration	46
Turtle	Decapitation	65
	Decerebration	58
Domestic hen	Arteriotomy	60
	Decerebration	53
Domestic duck	Arteriotomy	58
	Decerebration	40
Pigeon	Arteriotomy	72
	After breaking neck	59
Opossum, *Didelphys virginiana*	Before decerebration (ether)	79
	After decerebration (ether)	55
Mouse (white)	After decapitation	86
	After crushing head	73
Rat, *Mus norvegicus*	Arteriotomy	65
	Blow on head	54
Guinea pig	Arteriotomy	66
	Asphyxiation (ether)	58
	Blow on head	53
Rabbit	Arteriotomy (ether)	73
	Decerebration (ether)	61
	Electrocution (ether)	60
Kittens	Arteriotomy (ether)	70
	Decerebration (ether)	50
	Asphyxiation (ether)	58
Cat	Arteriotomy (ether)	66
	Asphyxiation (ether)	59
	Decerebration (ether)	44
	Electrocution (ether)	46

TABLE 1—*Concluded*

ANIMAL	METHOD OF SLAUGHTER	PHYTOTOXIC INDEX
		per cent
Dog	Arteriotomy (ether)	75
	Asphyxiation ether)	64
	Decerebration (ether)	48
	Femoral artery ligated before decerebration (ether)	63
Sheep	Arteriotomy (Šeḥiṭa)	65
	Blow on head	56
Calf	Arteriotomy (Šeḥiṭa)	60
	Blow on head	53
Ox	Arteriotomy (Šeḥiṭa)	61
	Blow on head	49

have their origin in the destroyed brain tissue was secured by the authors in experiments on cats and dogs in which the femoral artery of one leg was ligated before decerebration. When extracts of muscle tissue from such a ligated limb after decerebration were compared with similar extracts prepared from muscle tissue of the opposite limb, which was not ligated, it was found that the former was much less toxic. On the other hand, when the ligature was loosened after decerebration while circulation and respiration were still maintained so as to allow the blood to pass through the previously ligated limb, muscle extracts obtained from that member were found to be very toxic, thus indicating that poisonous substances had been carried to it by the circulation.

In case of electrocution experiments, another factor must undoubtedly be involved in the increased toxicity of the muscle extracts. In all such experiments the muscles exhibited prolonged clonic and tonic contractions, which involved the formation of abnormal metabolic products. These products probably explain the difference in toxicity between electrocuted muscle and normal controls.

It is well to state in this place that in all experiments in which the muscle tissues obtained after different modes of slaughter were compared, the age of the specimens was always the same. This was absolutely necessary because the authors have found that muscle tissue loses its toxicity to some extent in proportion to the time elapsing after killing of the animal, as was indicated by a daily study of the phytotoxic indices obtained from specimens kept for a number of days. This interesting change in the toxicity of muscle tissue occurs even when meat is kept in the ice-box at a low

temperature. A detailed study of the keeping qualities of meats or muscle tissues obtained after various modes of slaughter is in progress and will be reported in a separate place.

The results of the present investigation are of considerable interest from both the scientific and practical points of view. Thus, for instance, it is conceivable that the reactions of a decapitated animal and a decerebrated one may be different in relation to the pharmacodynamic responses in physiological and pharmacological experiments. It may be well to investigate whether the standard frog method for assaying digitalis will give different quantitative results when the animals are decerebrated or pithed, on the one hand, or decapitated, on the other. Again, the data obtained are obviously of considerable interest from the standpoint of wholesomeness of meats obtained by various methods of slaughter.

SUMMARY

1. Pharmacodynamic reactions of muscle extracts were studied after arteriotomy, asphyxiation, decerebration and other injuries to the brain, and after electrocution.

2. A large variety of animals, including representatives of amphibia, reptiles, fish, birds, marsupials and mammalia, was employed in this study.

3. In case of frogs, fish and turtles, muscle extracts obtained after decerebration were found to be more toxic than those prepared after decapitation of two individuals of the same species.

4. In all warm-blooded animals, extracts made from muscle obtained after arteriotomy and exsanguination were much less toxic than similar extracts prepared from muscle tissue after injury to the brain or decerebration *in situ*.

5. The difference in toxicity is undoubtedly due to the setting free in the circulation of toxic bodies produced by the trauma, which products are carried to the muscles by the blood stream before the heart stops beating.

6. In case of electrocution, the greater toxicity of muscle extracts, compared with normal controls, is probably due to products of abnormal catabolism resulting from the powerful clonic and tonic contractions of the muscles induced by the electric current.

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THE INFLUENCE OF SECTION OF THE CERVICAL SYMPATHETIC NERVE AND EXTIRPATION OF THE SUPERIOR CERVICAL GANGLION ON THE COMPOSITION OF THE PAROTID SALIVA IN THE DOG

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Henri and Malloizel (1902) and Babkin (1913) demonstrated that, after extirpation of the superior cervical ganglion in dogs with a permanent salivary fistula of the parotid or mixed glands, the flow of saliva, activated in a normal way, was richer in organic matter than before extirpation of the ganglion. This fact led Babkin to the conclusion that "trophic" impulses to the gland (in Heidenhain's sense) were not transmitted through the sympathetic nerve during reflex stimulation of the salivary secretion.

The four phase experiment on dogs with permanent salivary fistula (Baxter, 1931) opens up a new method of approach to the investigation of this very important problem and permits a clearer interpretation of the meaning of the double innervation of the salivary glands.

METHODS. Two dogs with permanent parotid fistula, described previously as dog 1 and dog 3 (Baxter, 1931) were employed in these experiments. In both dogs section of the vago-sympathetic nerve was performed aseptically, and later the superior cervical ganglion was extirpated in dog 1. The composition of the saliva, i.e., solids, organic substances, ash (Baxter, 1929, 1931) and chlorine, was investigated in the case of both dogs immediately after operation and at intervals during the succeeding months. The last investigation was performed on dog 1 twelve months after operation and on dog 3 ten months after operation.

Effect of section of the vago-sympathetic nerve. The following changes occurred in the composition of the saliva in both dogs shortly after section of the vago-sympathetic nerve.

The organic moiety was lower in phase 1A after section of the nerve than before. Whereas before section of the sympathetic nerve the concentration of organic substances on bread and meat powder in phase 1A was 85 to 95 per cent of the organic concentration in phase 1B, after section of the nerve it fell to about 50 or even 30 per cent of the former value. The total concentration of the organic moiety in phases 1A and 1B immediately after section of the vago-sympathetic nerve was practically the same as

before section. Some months later the total concentration of organic matter in phases 1A and 1B had risen considerably.

In phase 2 the organic moiety was higher after section of the nerve, while in phase 3 the amount did not vary significantly. In each phase the inorganic fraction before and after operation was almost identical. The same is true for the concentration of chlorine.

These relations may be observed in table 1, which comprises the average data from three bread and meat powder experiments on dog 3. (The quantities of saliva shown in tables 1 and 3 represent total secretion obtained during four periods of stimulation). They were carried out on March 27th, 28th and 29th (before the operation) and on April 2nd, 3rd and 5th (after the operation), the operation being performed on March 31st. Analogous data were obtained with dog 1.

TABLE 1

Dog 3. Bread and meat powder (average figures)

The dog was stimulated four separate times at fifteen-minute intervals.

PHASE	BEFORE OPERATION					AFTER OPERATION				
	Total amount	Sol.	Org.	Ash	Cl per 100 cc. saliva	Total amount	Sol.	Org.	Ash	Cl per 100 cc. saliva
	cc.	*per cent*	*per cent*	*per cent*	*mgm.*	*cc.*	*per cent*	*per cent*	*per cent*	*mgm.*
1A	4.4	1.9	0.46	0.73	287	4.2	1.03	0.32	0.71	296
1B	4.4	1.27	0.53	0.74	258	4.1	1.36	0.65	0.71	239
2	3.2	1.08	0.44	0.64	210	2.9	1.19	0.57	0.62	187
3	3.0	0.56	0.16	0.40	126	1.7	0.51	0.12	0.39	127

The changes in the distribution of the organic moiety in different phases of the parotid secretion on bread and meat powder could still be observed several months after sympathectomy, although they were not then quite so marked as immediately after the operation. Experiments were performed on dog 3 in June 1930 and January 1931. Comparison of all these results shows that before section of the nerve the average concentration of organic substances in phase 1A was 86 per cent of the amount in phase 1B, and immediately after the operation it was about 48 per cent, while three months after it was 45 per cent and ten months after 60 per cent. It was also found that in the later experiments there was a greater average concentration of organic substances in phases 1A and 1B, i.e., during the first minute of secretion. Thus the average concentration of the organic moiety in phases 1A and 1B before the operation was 0.49 per cent; immediately after the operation it was 0.48 per cent (table 1); three months later, 0.79 per cent; and ten months later 0.88 per cent.

Analogous results were obtained with rejectable substances, such as 0.25 per cent hydrochloric acid. In dog 3 before the operation, when hydrochloric acid was injected into the mouth, the concentration of the organic moiety in phase 1A was 79 per cent that of phase 1B. Ten days after the operation it was 31 per cent, four months after 32 per cent, and ten months after 48 per cent.

Experiments with pilocarpine. In both dogs (nos. 1 and 3) feeding or injection into the mouth of 0.25 per cent HCl during pilocarpine secretion gave analogous results. For a month after section of the vago-sympathetic nerve, reflex stimulation produced a much weaker effect than before section. Not only was the increase in the concentration of organic substances less

TABLE 2

Dog 3. 3 mgm. pilocarpine and bread and meat powder

The saliva was collected in five-minute periods.

EXPERIMENT MARCH 10				EXPERIMENT APRIL 9				EXPERIMENT JUNE 30			
Amount	Sol.	Org.	Ash	Amount	Sol.	Org.	Ash	Amount	Sol.	Org.	Ash
cc.	*per cent*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*per cent*	*cc.*	*per cent*	*per cent*	*per cent*
0.5	0.73	0.26	0.47	0.7	0.47	0.75	0.42	2.0	0.64	0.32	0.32
0.8	0.56	0.20	0.36	1.0	0.37	0.07	0.30	2.4	0.52	0.18	0.34
1.7	0.42	0.18	0.24	1.3	0.20	0.04	0.17	1.9	0.44	0.14	0.30
2.6	0.48	0.06	0.42	1.4	0.21	0.04	0.17				
1.5	0.42	0.08	0.34	1.1	0.21	0.05	0.16	*8.3	1.22	0.47	0.75
								2.0	0.87	0.26	0.61
*7.1	1.31	0.56	0.75	*8.7	0.91	0.29	0.62				
1.8	0.86	0.26	0.60	1.3	0.70	0.25	0.45				
0.4				0.6							
0.4	0.58	0.22	0.36	0.6	0.40	0.04	0.36				
0.4				0.2							

* Fed bread and meat powder at intervals during this 5-minute period.

during feeding, but the after-effect of stimulation of the mouth was shorter. In general after sympathectomy the saliva was poorer in all its constituents than before. Three months after the operation the gland reacted almost normally to the various stimuli. The chlorine concentration followed practically the same course as the ash (Baxter, 1930). The following experiments performed on dog 3 before and after section of the vago-sympathetic nerve illustrate these facts. (Table 2.)

Experiments with atropine. It was observed that the amount of saliva secreted by the atropinized gland after section of the sympathetic nerve was slightly less than before the operation. No definite variation was noted in the relationship between the organic and inorganic fractions after severing of the vago-sympathetic nerve.

Result of extirpation of the superior cervical sympathetic ganglion. On March 12, 1930, the left superior cervical sympathetic ganglion of dog 1 was extirpated. This dog, which had a left parotid fistula, had had the sympathetic nerve supply to the left parotid gland removed three months previously by severing of the corresponding vago-sympathetic nerve. After the removal of the ganglion, it was found that the concentration of inorganic salts was almost unchanged, but there were changes in the quantity of organic matter secreted and in the concentration of chlorides in the different phases. With regard to the organic fraction, experiments were performed on this dog during the period March 25, 1930, to January 1931. The concentration of organic substances in the average of phases 1A and 1B was found to be increased. (Table 3.)

The relation between the concentration of organic matter in phases 1A and 1B also changed. After section of the sympathetic nerve the concen-

TABLE 3

Dog 1 (for explanation see table 1)

AFTER SECTION OF VAGO-SYMPATHETIC NERVE					AFTER EXTIRPATION OF SUPERIOR CERVICAL SYMPATHETIC GANGLION				
Bread and meat powder, February 20, 1930					Bread and meat powder, January 5, 1931				
Phase	Total amount	Sol.	Org.	Ash	Phase	Total amount	Sol.	Org.	Ash
	cc.	*per cent*	*per cent*	*per cent*		*cc.*	*per cent*	*per cent*	*per cent*
1A	5.7	0.92	0.14	0.70	1A	5.4	1.12	0.38	0.74
1B	5.9	1.32	0.50	0.82	1B	6.6	1.59	0.83	0.76
2	3.9	1.32	0.50	0.82	2	4.1	1.61	0.78	0.83
3	2.8	0.90	0.28	0.62	3	1.8	0.97	0.39	0.58

tration of organic matter in phase 1A only amounted to one-third of that in phase 1B when bread and meat powder were given. After removal of the ganglion, the organic moiety of phase 1A rose to one-half the proportion of organic elements in phase 1B. These observations were confirmed when other stimuli were used.

The course of the chlorine secretion was likewise disturbed. In normal conditions as well as after section of the vago-sympathetic nerve, a steady fall in the concentration of chlorine from phase 1A to phase 3 was usually the rule. In a few instances after section of the sympathetic nerve the amount of chlorine secreted in phases 1A and 1B respectively was practically equal. After removal of the superior cervical ganglion the concentration of chlorine was for a time decidedly higher in phase 1B than in phase 1A. A few days after the operation the secretion rate of the chlorine had returned to normal.

Changes caused by extirpation of the superior sympathetic cervical ganglion were noted in experiments involving subcutaneous injection of pilocarpine and stimulation of the mouth cavity with food or with 0.25 per cent HCl. The volume of the secretion from the same amount of pilocarpine (2.5 to 3 mgm.) was somewhat greater than under normal conditions or after section of the vago-sympathetic nerve. (The total secretion was greater by 2 or 3 cc. after extirpation of the ganglion than when the innervation was intact or when only the vago-sympathetic had been cut.) The concentration of organic substances and ash was not lowered, as it was immediately after section of the vago-sympathetic nerve, but was throughout higher even than before any interference with the sympathetic nervous supply.

Discussion. The four-phase experiments on the parotid gland before and after removal of the sympathetic innervation enabled us to obtain a better understanding of the mechanism of the glandular reaction. Two hypotheses concerning the greater concentration of the organic moiety in the second phase were put forward in a previous paper (Baxter, 1929), where it was suggested that it might be due either to central influence or peripheral mechanism. The second, however, must be excluded. The rise in the concentration of the organic substances in phase 1B and phase 2 could not be due to the variation of the tonus of the blood vessels, since variation was also observed in glands completely deprived of their sympathetic innervation. It must therefore be attributed to the varying state of excitation of the salivary centre.

Separate analysis of the composition of the saliva in phases 1A and 1B furnished a clue to the comprehension of the rôle played by the sympathetic nerve in the process of salivary secretion. Whereas under normal conditions the concentration of the organic moiety in phase 1A in both dogs investigated was a little less than in phase 1B (bread and meat powder), after section of the sympathetic nerve this relationship changed entirely. The quantity of organic elements in phase 1A was much lower than normal, and in phase 1B was somewhat higher than before the operation, so that the total of the organic fraction in the saliva secreted during the first minute was practically unchanged. Therefore, as the lessened quantity of organic substance in phase 1A after vago-sympathectomy cannot be attributed to loss of trophic impulses, which were conveyed to the gland along the parasympathetic nerve, another explanation was sought. The work of other investigators in this laboratory (MacKay, 1927, 1929; Babkin and MacKay, 1930; Stavraky, 1930) proved that in addition to secretory and vasoconstrictor fibres the sympathetic nerve supplies the salivary glands with motor fibres which during stimulation press out the contents of the glands. A possible explanation of facts observed in this investigation is that after section of the sympathetic nerve the parotid gland no longer re-

ceives the special reflex motor impulses. The diminution in the organic elements in phase 1A after section of the sympathetic nerve may conceivably be due to the fact that granules accumulated in the cells were not pressed out during the initial flow of saliva.

Further, the secretion of water and salts initiated by the parasympathetic, so-called "secretory" effect of Heidenhain possessed a shorter latent period than the accumulation of organic matter in the saliva (the so-called "trophic" effect of Heidenhain). There is therefore a slower accumulation of organic substances in the saliva of a gland deprived of its sympathetic nerve supply and a higher concentration of organic constituents in phase 1B or in the after-flow of phase 2. From the third to the seventh minute (phase 3) there is gradual fading of both processes (i.e., secretory and trophic), and this explains why the secretion in this period is scanty in volume and poor in organic elements.

The reaction of the parotid gland to atropine after section of the vago-sympathetic nerve and extirpation of the superior sympathetic cervical ganglion was no different from that of normally innervated glands. There was, however, a marked difference in the reaction to pilocarpine of glands deprived of their sympathetic innervation. Immediately after section of the vago-sympathetic nerve there was a great fall in the concentration of organic matter in the pilocarpine saliva, and during the period of stimulation of the mouth cavity with food or rejectable substances the secretion increased somewhat in volume. In the course of two or three months there was a gradual restoration of the normal relations. After extirpation of the superior sympathetic cervical ganglion, there was a marked increase in the total volume of the secretion from the parotid glands and a definite rise in the concentration of the organic moiety.

After extirpation of the superior cervical ganglion, parotid saliva activated by the usual stimuli (bread and meat powder, 0.25 per cent hydrochloric acid, etc.) was also somewhat richer in organic matter than normal saliva or that obtained after section of the vago-sympathetic nerve. These data are in accordance with the observations of Henri and Malloizel and of Babkin. Although no control experiments were performed by these investigators or by the present writer, certain data indicate that removal of the superior cervical sympathetic ganglion permanently increases the flow of blood through the salivary glands, and hence increases the nutrition of the glandular cells. Bradford (1888) found that, three months after extirpation of the superior cervical ganglion in the cat, both the submaxillary and the parotid gland were heavier than on the normal side. Anrep and Kahn (1924) noted that the restoration of mucin in the exhausted submaxillary gland was more rapid after extirpation of the ganglion than before. This is further evidence that increased vascularization of the gland leads to optimum nutrition of the secretory elements. Thus even after section of the

cervical sympathetic nerve the superior cervical sympathetic ganglion sends tonic impulses to the blood vessels of the salivary glands. From this viewpoint may be explained the greater concentration of organic substances in saliva secreted by reflex stimulation after extirpation of the ganglion. The impulses which reach the gland through the parasympathetic nerve act on the glandular cells, which have an opportunity of accumulating more organic material than the normal gland.

One other fact must be mentioned. There was a definite increase in the volume of the secretion after extirpation of the superior sympathetic cervical ganglion, which was most clearly observed in the experiments with pilocarpin. This was probably due to greater vascularization of the gland, the blood vessels of which had lost their tone after complete sympathectomy.

SUMMARY

1. The rise in the concentration of organic substances in phase 1B and phase 2 of the parotid secretion is to be attributed to the varying state of excitation of the salivary centre and not to changes in the vascularization of the gland, since this was observed after the removal of the sympathetic innervation of the gland.

2. After section of the sympathetic nerve to the parotid gland, the initial output of the organic constituents (phase 1A) was lower, the concentration of salts remaining unchanged.

3. It is suggested, as a possible explanation of this fact, that after section of the sympathetic nerve the parotid gland no longer received the special motor impulses transmitted to it reflexly through this nerve.

4. Immediately after section of the vago-sympathetic nerve there was a great fall in the concentration of organic matter in the pilocarpine saliva. In the course of two or three months there was a gradual restoration of the normal relations.

5. After extirpation of the superior cervical sympathetic ganglion, the relatively lower concentration of organic substances in phase 1A as compared with phase 1B, was more pronounced. The absolute concentration of organic matter in the saliva after extirpation of the ganglion was higher than normal, as a result probably of better blood supply to the gland.

6. After extirpation of the superior cervial sympathetic ganglion, pilocarpine activated a more profuse secretion, having a higher concentration of organic substances.

The writer wishes to thank Dr. B. P. Babkin, under whose direction this investigation was carried out, for his very helpful advice and criticism.

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THE COMPENSATORY PAUSE

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The demonstration of an "extra" or "premature" beat of the frog's ventricle in response to an adequate stimulus applied during the phase of diastole is a common laboratory experiment. With respect to this particular phenomenon the ventricular cycle may be divided into the absolute refractory period, during which no stimulus can elicit a response, the relative refractory period during which irritability returns and strong stimuli may be effective, and the responsive period. The responsive period corresponds roughly to the phases of relaxation and rest or diastasis, and ends abruptly with the beginning of the succeeding ventricular systole.

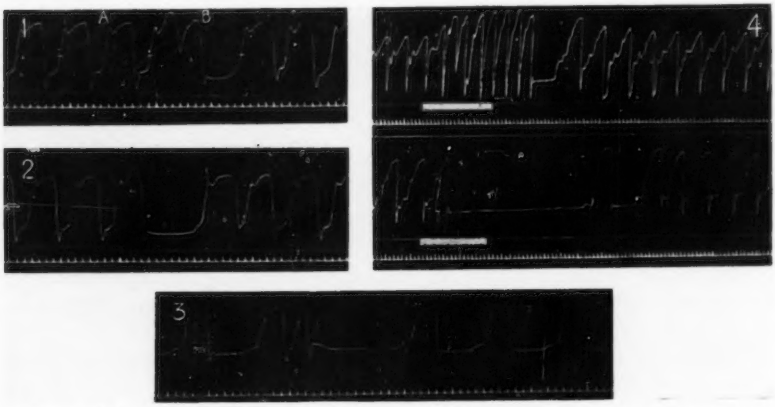
When such a "premature" beat has been induced there follows a pause which, because it is of such duration as to restore approximately the original rhythm, is known as the compensatory pause (Marey, 1876). Much work has been expended on the exact determination of the refractory period, on the relatively refractory period, and on the vagaries of the induced beat, and very little on the pause though it seems to be of fundamental importance since it may have some relation to those other states in which tissue activity is temporarily in abeyance: the refractory period, diastasis—using the word in Henderson's sense (1906)—or even inhibition itself.

The usual explanation for the compensatory pause, as formulated by Gaskell (1900), is that the rhythmical contractions of the pace-making sinus venosus are not disturbed by stimulation of the ventricle, but that one of the contraction waves of the sinus, carried over to the auricles, is blocked by the ventricle which is then in the refractory, systolic phase of the induced beat. It is thus not until the next regular beat of the sinus that a full beat of the entire heart, sinus venosus, auricles, ventricle and bulbus, can take place. It is thus assumed that the sinus is not affected and that the sequence of beats is accurately preserved.

We have undertaken a study of the compensatory pause to determine its variations and, if possible, the causes that are responsible for them. In our experiments the contractions of the heart are recorded by the suspension method and the heart is stimulated through long slender nickel-plated spring wires in contact with the surface and so flexible that they follow without unduly interfering with the movements of the heart. Un-

less otherwise noted, the stimulus, a single make induction shock, is applied at a constant point on the descent of the lever by causing a wire attached to the lever to dip into a mercury-filled container of large area and adjustable level, thereby completing a circuit through an electro-magnet which in turn closes and locks the primary circuit of the stimulating induction apparatus.

Where the time of stimulation is to be varied the same electro-magnet sets in motion a simple delay apparatus by which the actual application of the stimulus may be deferred at will, as in the more frequently used pendulum keys.



Figs. 1, 2, 3 and 4. 1. Induced beat and compensatory pause, showing the method of measuring. 2. A compensatory pause without extra beat of the ventricle, following early stimulation of the auricle. 3. Reversed beat in a heart slowed by atropin. 4. The effects of stimulation of the crescent in the normal heart and in the same heart treated with ergotamine tartrate.

Thus we have been able to duplicate stimuli with great accuracy or, at will, to explore the responsiveness of the heart during the complete cardiac cycle.

We have found it difficult to measure the duration of the pause alone with any degree of accuracy. We have therefore measured the time between corresponding points of the beat preceding the premature contraction and the next succeeding one (the B group in fig. 1). This measurement is compared with a similar measurement for the two preceding regular beats (the A group). From this comparison we have at least a fair indication of the degree to which compensation is achieved, and it is inferred that any variation in the length of the B group is due chiefly to a variation in the pause.

There are many difficulties in such a method. It is not always possible to

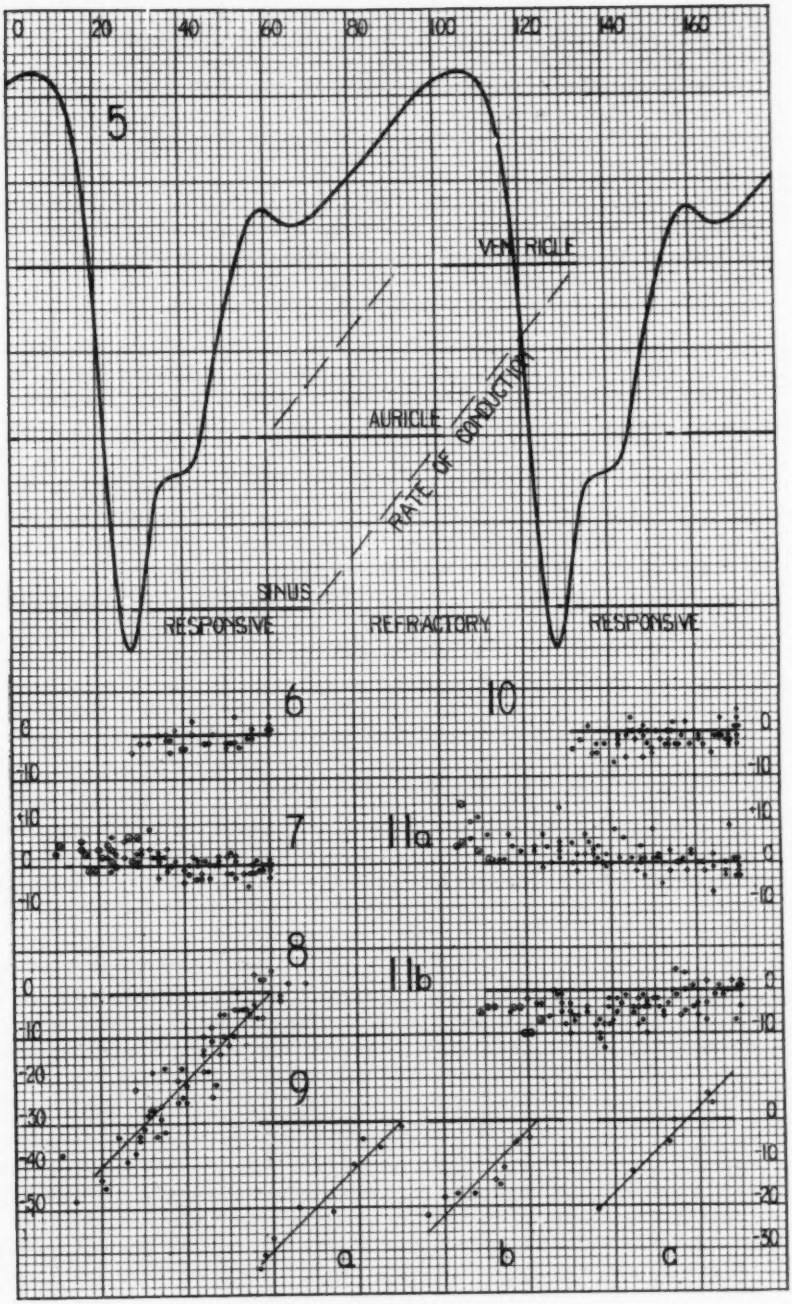
select definite corresponding points, but in that case a horizontal line is drawn through the most constant part of the up-stroke of the lever, and the distance between these intersections is measured. Again, the A group is itself not constant but varies frequently by one or two, occasionally by as much as three per cent. Typical figures are, for example, 187, 185, 186, 185, 189, 186 hundredths for a consecutive series of measurements of the A group, taken at regular intervals with surrounding conditions constant. However, data obtained from a sufficiently large number of accurate measurements yield results that are definite enough to be reliable.

Figure 5 represents in composite form the responsive periods from five consecutive hearts. The whole cardiac cycle was explored not only for ventricular extra beats, but for those obtained from the auricles and the sinus as well. On the upper level of the chart is represented the responsive period of the ventricle, on the second level the responsive period of the auricles and on the lowest level that of the sinus venosus, each in its relation to the cardiac cycle.

It will be noted that since the end of the responsive period in each division of the heart marks the beginning of its systole, we can infer with some accuracy the time of the initiation of the beat in sinus, auricles and ventricle. We find that the time elapsing between the beginning of the sinus beat and the beginning of the auricular beat is approximately the same as that elapsing between auricles and ventricle. The interval varies from 24 to 36 hundredths of a second in different hearts. With a heart rate of sixty to the minute the interval is ordinarily 32 to 34 hundredths. This constancy in the rate of conduction from sinus to auricles to ventricle has characterized all of our experiments. The rate of conduction can be expressed in the chart by an oblique line joining the three points referred to.

By measuring in the manner described the duration in hundredths of a second of the two cycles including the compensatory pause (B group)

Figs. 5, 6, 7, 8, 9, 10 and 11. 5. The responsive periods of the normal ventricle, auricles and sinus, a composite from five hearts. The scale represents hundredths of a second. 6. The variations in compensation after an extra beat of the ventricle. The base line represents the responsive period. Lengthened measurements, indicating retardation of the succeeding beats, are plotted above the line. Measurements plotted below the line are shortened and indicate advance of the succeeding beats. The scale shows hundredths of a second. 7. The degree of compensation after extra beats of the auricles, similarly plotted. 8. The degree of advancement of the sequence following stimulation of the sinus. 9a, b and c. Types of charted results obtained by stimulation of the sinus. See text. 10. The degree of compensation in the ergotaminized ventricles of five hearts. 11a. The variations from compensation shown by five normal hearts when the stimuli were applied to the auricles during the responsive period. 11b. The variations shown by the same five hearts when stimulated during the auricular responsive period after treatment with ergotamine tartrate. The scale shows hundredths of a second.


0
20
40
60
80
100
120
140
160
5
VENTRICLE
AURICLE
RATE OF CONDUCTION
SINUS
RESPONSIVE
REFRACTORY
RESPONSIVE
6
7
8
9
10
11a
11b
a
b
c

and comparing each measurement with that for the previous two beats of the regular rhythm (A group) we have accumulated a large mass of data which might be treated statistically, but is more easily interpreted when presented in graphic form.

Taking up the three subdivisions of the heart in detail, we find that the ventricular responsive period is of briefer duration than in the case of auricles and sinus; and for the ventricle we find that the duration of the B group is shorter with the earlier applications of the stimulus. Figure 6 represents the measurements obtained from five consecutive hearts where stimulation induced a premature beat. The horizontal line represents the responsive period. Each dot represents a test and its measurement. The position of a dot along the horizontal line indicates the time of application of the stimulus in relation to the cardiac cycle. The position of a dot above or below the horizontal line represents the degree of lengthening or shortening of the B group observed when the stimulus was applied at that time. Measurements plotted above the line indicate lengthening; below the line they indicate shortening. The scale used in plotting the chart takes into account hundredths of a second.

It is evident that the degree of compensation changes. There is certainly no invariability such as one might expect if we assume that the sinus absolutely controls the rate of succession of the beats. The sinus need not be affected by ventricular stimulation and the Gaskell explanation of the compensatory pause assumes that it is not affected. There is, therefore, some as yet undetermined factor influencing the degree of "compensation" and the restoration of the regular rhythm of the ventricle after a premature beat. It may be that the variation is to be ascribed to the damming back of the blood stream by the premature beat, with a resulting widening of the next contraction wave and some encroachment on the space representing the pause. In that case the earlier extra beats would leave more blood in the sinus and auricles, and would show the greater shortening effect.

In the case of the "extra," "premature" or induced beats of the auricles, plotted in figure 7, there is an even more marked departure from true compensation. The first part of the auricular responsive period shows the longer deviations, in some cases amounting to as much as ten per cent of the duration of a single cardiac cycle, a very considerable variation from exact compensation. Here there seems to be no adequate explanation in mere blocking of contraction waves for the marked variation in the duration of the B group. It can hardly be the damming back of the blood stream by the aborted beat, with a resulting enlargement of the contraction wave that follows the pause. That explanation might serve for the ventricular measurements, but the same explanation cannot hold true for the auricular extra beats, for it is the earlier auricular beats that are followed by a lengthening of the B group, the measurement decreasing with the later

stimuli, while for the ventricle the measurement increases as we move to the right and apply the stimulus later.

In the early part of the responsive period of the auricle one finds the cases in which a compensatory pause occurs without an extra beat of the ventricle, as in figure 2. The measurements of such cases are indicated by circles in the chart. As Porter (1900) and Ishikawa (1924) have pointed out, this result is obtained when the excitation proceeding from the auricles reaches the ventricle during its refractory period. Such a possibility is obvious if we refer to our chart of the responsive periods (fig. 5), bearing in mind the already determined rate of conduction from auricle to ventricle. It will be seen that contraction waves originating in the early part of the auricular responsive period will reach the ventricle during the latter part of its refractory period as shown by the broken line. The stimulus causes an induced beat of the auricle, which is extinguished when it reaches the still refractory ventricle. The refractory period of the induced auricular beat in turn extinguishes the next autochthonous sinus beat. There is thus no beat of the ventricle until the sinus beats again and the wave of contraction can proceed normally through the auricles to the ventricle.

Such an observation has an added interest from the fact that the ventricle is quiescent for such an unusual length of time. If the heart is beating with a rhythm of 60 to the minute, then the sinus responds to its inner stimulus at intervals of one second, and sets the pace for the auricles and ventricle. It is assumed that the irritability of the ventricle is less and that its autochthonous rhythm would be slower. In the case of a prematurely beating ventricle the time between beats may be as much as 1.3 second, and perhaps such an interval may not be too great for the ventricle to remain quiescent. But in the cases just described, when a compensatory pause occurs without a ventricular extra beat there is an interval of two seconds, twice that between the autochthonous sinus beats. It is problematical whether such an interval of quiescence can be accounted for by citing the assumed irritability gradient of the heart, without invoking some degree of inhibition as a possible contributing factor.

Turning now to the sinus (fig. 8) we find that stimulation is followed not by an extra beat of the sinus alone, but by what Starling (1926) describes as an advance of the sinus beat and of the whole sequence. There is no question of blocked contraction waves, but instead a shortening of the duration of the B group that is quite characteristic and predictable. By just as much as the artificial stimulus anticipates the autochthonous beat there is an advance of the sequence and a shortening of the measurement of the B group. The points plotting these shortened measurements will thus tend to lie along a line that falls farther below the base line as the stimuli are applied earlier. Since the time scale is the same in both directions in our chart, all measurements for premature beats should fall on a

line intersecting the base line at the end of the responsive period and at an angle of 45° to the base line, as shown in the chart.

In formulating this statement two assumptions are made. First, we take it for granted that the latent periods are the same for autochthonous and stimulated sinus beats, a small matter at best, but one that we have no method of testing. Second, we assume that there are no new factors influencing the measurement of the B group. There may, however, be at least two such factors. If, for example, the stimulating electrodes are so placed on the sinus that there is an additional delay in starting an artificially induced beat because of time lost in transmitting the contraction to its usual site of origin, then for just that length of time there will be no advance of the sequence and therefore no shortening of the measurement of the B group. The plotting of the results of stimulation of the sinus would be in that case along the base line to the left of the point of origin of the autochthonous beat, and only the earlier stimuli would show the typical advance, as in figure 9b. We have thus, by shifting the electrodes until the results conform to the type shown in figure 9a, a trial and error method for determining the site of origin of the normal beat, the "sinus node," if one may use that term. From such results as we have accumulated we conclude that this "node" lies in or near the sino-auricular junction.

A second factor that may modify the charted results of stimulation of the sinus is inhibition. If the stimulus that causes the premature beat of the sinus also stimulates the inhibitory apparatus, then presumably all measurements will be lengthened by the same increment, and the plotted results of stimulation throughout the responsive period will lie in a straight line, but in a line elevated above its position were inhibition not involved. Such a record is shown in figure 9c.

We have suggested the possibility of an inhibitory factor in the duration of the compensatory pause. It is more nearly correct to suggest a nervous factor, the effect of which should be to lengthen or shorten the pause according to whether the stimulus applied directly to the heart affects more the inhibitory or the accelerator fibers among the intrinsic nerves of the heart.

To test for this nervous effect we have resorted to various expedients. 1. Strong stimuli have been alternated with weak on the assumption that the stronger stimuli would give the greater effect. There has been no constant result, though in most cases the B group (compensatory pause) is of longer duration where the stimulus is strong. 2. We have tested the effect of multiple stimulations, using two, four, six, eight or ten make and break induced currents to elicit the extra beat. In this case also the results were inconstant. 3. We have tried the effect of applying stimuli during the refractory period, both for single stimuli and for series of five stimulated

beats, only to find that the duration of the succeeding unstimulated beats may be increased consistently, or just as consistently shortened.

The results that we have obtained by these methods can be explained on the assumption that there is an effect produced through stimulation of the intrinsic nerves, and that the effect varies with the relative effectiveness of the accelerator and inhibitory nerves at the time of the experiment.

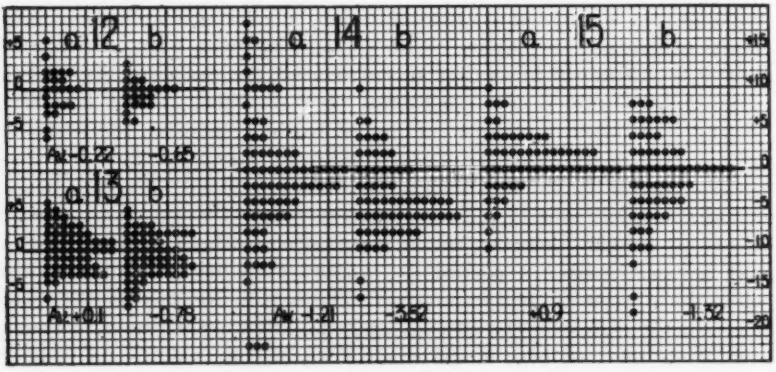
Acting on the hypothesis that nerves of both kinds may be concerned we have attempted to eliminate the inhibitory nerves by the use of atropin, which is credited with the power to abolish the effect of stimulating the cardio-inhibitory fibers. We have found the side effects of the atropin, in amounts that will prevent cardio-inhibition, so great that we have no successful experiments to report. With atropin, however, when the characteristic slowing of the heart is at all pronounced, one can study the effects of stimulation under new conditions. For example, in the normally beating heart when a stimulus is applied to the responsive ventricle the "extra" beat of the ventricle is prevented from straying beyond the ventricular borders by the fact that the auricles are in the refractory phase during the whole time of the ventricular responsive period. In the heart slowed by atropin, however, the long period of diastasis in both auricles and ventricles makes it possible to produce extra beats in the venticle while the auricles are still responsive. The contraction wave in that case is conducted back over the auricles, and similarly over the sinus, giving a completely reversed beat of the whole heart (fig. 3). While the orderly progress of a normal contraction is no doubt determined by the irritability gradient, the long refractory period in each division of the heart serves as a useful and perhaps necessary check in preventing irregularities or reversal under ordinary conditions. The pause following the reversed beat is determined chiefly by the rhythmicity of the sinus. The sinus will beat again at a time determined by whatever factors are controlling its rhythm when the stimulus is applied.

Failing thus to separate the two antagonistic nervous effects by the use of atropin, we have attempted the complementary procedure and have poisoned the accelerator fibers by the use of ergotamine tartrate.[1] While the dosage must be large (1 cc. of a 0.01 per cent solution injected slowly into the ventricle) and while the full effect may not appear for thirty or forty minutes, we have been able to abolish accelerator and augmentor effects of stimulation of the crescent, even to the extent of eliminating the rapid beats that usually result from crescent stimulation during the actual application of the stimulus (fig. 4). (This would seem to imply that these rapid beats, usually ascribed to direct effects on the heart muscle, may be dependent on the presence of augmentor nerves.)

[1] The preparation used was kindly supplied in convenient form by Sandoz Chemical Works, Inc.

In these experiments, apparently complete elimination of the augmentor and accelerator nerves has been obtained without any impairment of the heart muscle. In fact the heart beats are improved, in that they are more regular and not so subject to the slight variations that we have referred to earlier.

Having thus succeeded in obtaining preparations in which only one type of nervous influence remains, we have repeated our series of measurements to determine what effect is produced by the elimination of the accelerator mechanism.



Figs. 12, 13, 14 and 15. 12. All measurements from five ventricles stimulated during the refractory period, *a*, before and *b*, after treating with ergotamine tartrate. Scale shows hundredths of a second. 13. All measurements obtained from stimulation of the left auricle during the late refractory period *a*, in five normal hearts and *b*, in the same five hearts treated with ergotamine tartrate. 14. All measurements from five ventricles stimulated during the refractory period for five successive beats *a*, before and *b*, after ergotamine tartrate. The scale shows hundredths of a second. 15. All measurements from five hearts following stimulation of the left auricle during the late refractory period for five successive beats *a*, before and *b*, after treating with ergotamine tartrate.

The ergotaminized ventricle shows no great change (see fig. 10). Throughout the responsive period, as in normal hearts, the early stimuli result in a shortening of the B group, the later ones showing progressively more nearly exact "compensation." The shortening is slightly more marked than in the normal heart (fig. 6).

We have found in our later experiments that where stimuli are applied to the normal ventricle during the refractory period, the result varies with the time of application. Applied just before the responsive period, in what is presumably the relatively refractory period, the effect is to shorten the duration of the next succeeding beats. This would seem to dispose of the

possibility that the variation from compensation where premature beats are elicited may be due to damming back of the blood stream, for there is no interruption of the regular flow of blood when the stimulus falls in the refractory period. Figure 12a shows the results from such stimulation of the normal ventricle and the increased shortening that is obtained from the same preparations when ergotamine tartrate is used (12b). When five stimuli are applied in the same way for five successive beats the increased shortening effect after ergotamine tartrate is used is even more evident (fig. 14a and b). It would seem to be true that the responses of the ventricle are only slightly affected by the elimination of the augmentor nerves.

In the case of the auricles the results are more striking (fig. 11). In normal hearts stimulation of the auricles during the early part of their responsive period gives a definite lengthening of the duration of the B group, compensation approaching more nearly to exactness the later the stimuli are applied. After the injection of ergotamine tartrate the result is reversed, and the early stimuli give B groups (compensatory pauses) that are shortened.

Again, in the case of the auricles single stimuli applied within three-tenths of a second before the responsive period begins, and therefore presumably in the time that includes the relatively refractory period, give lengthening of the B group in the normal heart (fig. 13a), and shortening in the same hearts after ergotamine tartrate has been injected (fig. 13b). Similarly, the effect of a series of five stimuli applied to successive beats gives lengthening of the stimulated group in normal hearts, shortening in hearts treated with ergotamine tartrate (15a and b).

It is thus apparent that the evidence already presented for a nervous factor in the determination of the length of the compensatory pause in the case of the ventricle is reinforced by even stronger evidence in the case of the auricles.

DISCUSSION AND SUMMARY

When an extra beat is elicited from the frog's heart the subsequent "compensatory pause" is not truly compensatory. After a ventricular extra beat there is some shortening of the pause, while for the auricles there is an equally constant loss of time and failure of compensation in the manner described.

These effects are not due to mechanical interference with the flow of blood for they are opposite in character in ventricle and auricles in normal hearts; and similar variations may be demonstrated even when the stimulus is applied late in the refractory period without interrupting the sequence of beats.

Further evidence for a nervous cause for these variations is found in the fact that while the shortening effect cited for the ventricle is slightly in-

creased when the heart has been fully ergotaminized the effect on the auricles is reversed.

Assuming that a nervous factor is to be recognized in the determination of the duration of the compensatory pause there are two findings still to be explained. First, we have the fact that the normal auricles, presumably innervated by both sympathetic accelerator and parasympathetic inhibitory fibers, show delay in compensation while the ergotaminized auricles, with only the inhibitory nerves functioning, show a shortening of the duration of the pause. It would seem that the opposite effects might have been expected. For the results as they actually appear we can suggest the possible explanation that we may have here another evidence of the anabolic action of the parasympathetic inhibitory fibers. It is more

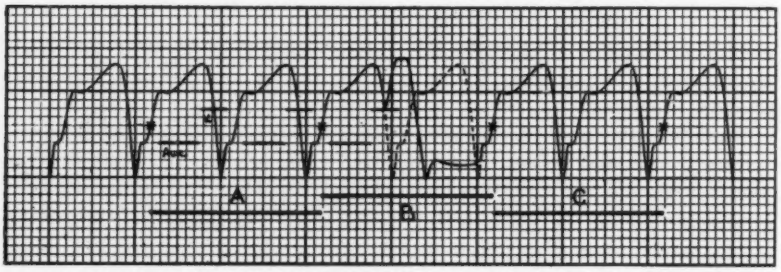


Fig. 16. Diagram showing the relation of the auricular, *Aur.*, and ventricular *V.*, responsive periods to the two cycles containing the compensatory pause, measured as the B group.

probable that both accelerator and inhibitory nerves when stimulated by a single induction shock cause a preliminary transient effect opposite in character to that which results later and with longer stimulation.

The second point, the contrast between auricular and ventricular effects, seems to present less difficulty. In the normal heart stimulation early in the auricular responsive period causes delayed compensation while the effect in the case of the ventricle is to shorten the pause. If we consider the ergotaminized heart in which only the parasympathetic inhibitory fibers are effective we note that the greatest shortening follows the stimuli that are applied early in the responsive period of the auricles (fig. 11b) and that in general the effect becomes less the later the stimuli are applied, and still less when applied to the ventricle (fig. 10). When once we admit that the effect of the intrinsic nerves in the ergotaminized heart is to quicken the immediately succeeding cycles we can see (fig. 16) why a stimulus applied early in the auricular responsive period should produce the maximum effect for it acts during almost the whole of the two cycles

that contain the compensatory pause. Later stimuli act for progressively shorter periods until at the end of the ventricular responsive period little more than one of the two measured cycles of the B group remains to be affected by the nerves. If we postulate a latency of about one second we can thus explain why stimuli applied late in the ventricular responsive period produce no quickening until after the compensatory pause is ended.

In the normal heart on the other hand, with sympathetic nerve supply intact and functioning, we assume that the effect of the sympathetic overcomes that of the parasympathetic where the two are acting together. The latent period of the sympathetic supply is, however, longer. It can control the duration of the pause only when the stimulus is applied early, and thus is effective only for the auricles. Since stimuli applied to the ventricles can act for only slightly more than one cycle the effect of the sympathetic on the ventricular series of measurements is slight, and appears only as a reduction in the degree of shortening that is produced by the parasympathetic.

In support of this explanation we have shown that when the usual weak single induction shock is applied to the normal ventricle and the pause is shortened in the usual way, the application of very strong single induction shocks gives less shortening, presumably because strong stimuli are more effective in exciting the sympathetic fibers. Thus the average for two hundred tests applied to fourteen hearts mid-way in the ventricular responsive period is a decrease of 1.18 hundredths of a second with weak and 0.42 hundredths of a second with strong stimuli. In ergotaminized hearts with the sympathetic control eliminated the corresponding shortening (average of fifty tests in each case) is 2.47 hundredths of a second for weak and 3.0 hundredths of a second for strong stimuli.

Again, we have numerous series of measurements showing that when the normal ventricle is stimulated in the responsive period the measurements of the B group show the characteristic shortening but the measurements of the next two beats (the C group in fig. 16) are lengthened.

It might also be pointed out that an exactly similar difference in latency is usually offered as one explanation for the "reaction" that follows inhibition of the frog's heart by simultaneous stimulation of the parasympathetic inhibitory and sympathetic accelerator fibers in the vago-sympathetic nerve.

It would thus appear that when an extra, "premature" or induced beat is elicited by the application of a single induction shock to either the ventricle or the auricles of the frog's heart, the duration of the "compensatory pause" is affected in the direction of lengthening by stimulation of the intrinsic sympathetic nerves, and toward shortening by stimulation of the intrinsic parasympathetic fibers. The resultant effect though in general predictable will depend on drug actions, on the strength of the

stimulus, on the time of application of the stimulus, on the relative latency of the two contrasted nerve supplies, in short upon anything that will affect their relative dominance at the time of the observation.

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FURTHER OBSERVATIONS ON EXPERIMENTAL AORTIC INSUFFICIENCY

IV. Hemodynamic Factors Determining the Characteristic Changes in Aortic and Ventricular Pressure Pulses

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While recent experimental evidence indicated the necessity of a frank reversal of previous opinion as to the magnitude of the regurgitation in aortic insufficiency, it left many hemodynamic problems to be explained. What determines the variation in the quantity of blood regurgitating in experiments performed under apparently similar conditions? Is it affected by the size of leak, the duration of diastole and the arterial pressure? What prevents the regurgitation of the entire systolic discharge? Why, if a considerable regurgitation occurs, does the relaxation gradient of the left ventricle remain unaltered? During what phase of relaxation does maximum regurgitation take place? If the maximum regurgitation occurs during isometric relaxation, during which phase there is normally no ventricular cavity, what forces are involved in creating a cavity?

In order to answer such questions and to evaluate the effects on pressure pulses produced by operation of physical laws and by simultaneous changes in physiological responses of the heart, the effects of experimental aortic leaks of different sizes were reinvestigated.

Procedures. Aortic and left ventricular pressure curves were optically recorded from anesthetized dogs. The technique described elsewhere (Wiggers, 1928) was followed, so attention need be called only to certain particulars. In order to give space for the instrument used to produce insufficiency the tip of the aortic cannula was placed 1 to 1.5 cm. distal to the aortic valves and not in close proximity as in our usual practice. This necessitates slight corrections in time relations of ventricular and aortic curves to allow for the velocity of pressure transmission. Records were taken on more rapidly moving film than previously in order to bring out clearly all changes in gradients. The heart was slowed somewhat either by clamping the S-A node or by mild vagal stimulation so as to produce cycle lengths comparable to those found in man, and to allow ample time

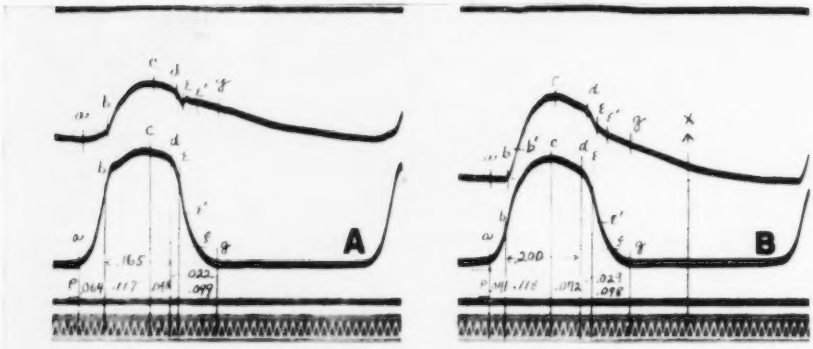

FIGURE 1

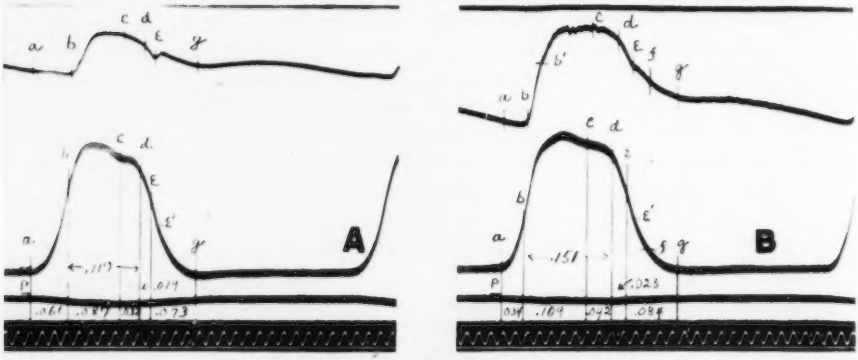

FIGURE 2

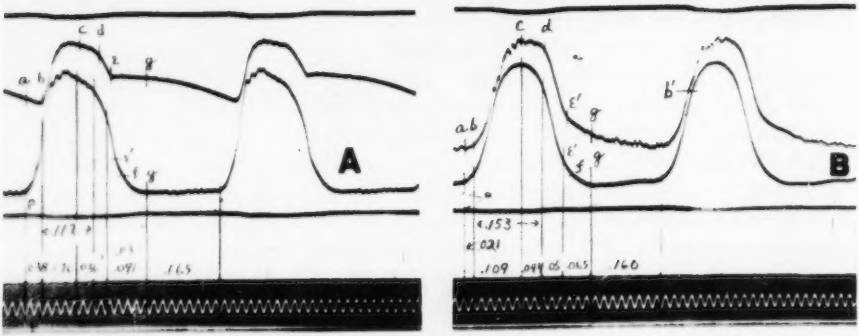

FIGURE 3

for completion of regurgitation. These procedures also obviated changes in heart rate which otherwise are apt to occur after production of a lesion and which unnecessarily complicate the analysis of results.

In the earlier experiments of this series, aortic leaks equal to from one-half to three-quarters the diameter of the aortic orifice were produced by use of a trocar previously described (1915). A study of records so obtained convinced us, however, that it is difficult by this method to equal the large sized leaks sometimes found in patients. The valves were still able to close around the tube which connected the aorta and the ventricular cavity, and the tube itself offered some resistance.

A new instrument, designated a *valve spreader*, was therefore devised. The distal ends of a series of fine wires about 3 cm. long were attached to a rod and their proximal ends to a movable sleeve sliding over the rod. By means of a screw arrangement in a handle these wires could be made to bulge at their centers to varying degrees. The instrument was introduced through the ventricular muscle into the aortic aperture in the closed position and in this state the valves closed effectively about it. With the instrument in position, the wires were gradually spread apart until with full expansion the valves were firmly held back against the wall; in fact the aortic ring could be dilated somewhat if the animals were not too large.

A large number of experiments were carried out on more than 24 dogs but some were not adapted to this analysis and others proved technically unsatisfactory, owing to the complicated character of the experiments. From these experiments 39 curves were selected as suitable for the type of analysis required. These curves were taken from experiments on 9 different dogs.

Analysis of records. While the custom of dividing the pressure pulses at the maximum peak into ascending or anacrotic and descending or catacrotic limbs has proved useful in relating graphic features to palpatory sensations, such a division is unsuitable as a basis for understanding the cardiodynamics of aortic regurgitation. Such an understanding can be attained only by dividing the arterial pressure pulse at the incisura into

Figs. 1, 2 and 3. Aortic (upper) and left ventricular pressure curves (lower) with respective base lines and time in 0.02 second, showing effects of size of leak. Segments A, normal condition; B, during insufficiencies at same heart rates. Figure 1 illustrates effects of moderate leaks (expt. C-491, VII); figure 2, effects of rather large leaks (expt. C-489, VI); figure 3, effects of maximal leak (expt. C-521, III-IV).

Lettering as follows: *a-b*, isometric contraction; *b-c*, maximum ejection phase; *c-d*, reduced ejection phase, also period during which true collapse could occur; *d*, end of systole; *d-e*, incisural decline of pressure; *e-f*, strict isometric relaxation; *f*, approximate time for opening a-v valves; *e-g*, post-incisural pressure decline. Note particularly changes of pressure gradients at *e*, *e'*, *f* and *g*, also abbreviation of isometric phase and prolongation of ejection and total systole with different sized leaks. Full discussion in text.

systolic and diastolic portions. We shall therefore discuss the systolic and diastolic alterations produced by leaks of different sizes and incidentally direct attention to changes in the ascending and descending limbs.

Typical aortic and ventricular pressure curves recorded before and after experimental production of moderate, large and very large aortic leaks are reproduced in figures 1, 2 and 3. The successive phases of systole and diastole are marked off by lines and the calculated duration of each phase is directly inscribed on the records. The cycle lengths before and after each lesion are not significantly changed.

Changes in the systolic portions. A glance at the aortic pressure pulses recorded after production of different sized leaks (figs. 1, B, 2, B and 3, B) reveals many similarities. The amplitude is larger than normal, the ascent is steeper, the systolic pressure top usually exceeds the normal and irregular vibrations are frequently superimposed on the basic pressure patterns. In addition, the decline during the last portion of ejection (*c-d*) occurs more rapidly. But the size of the leak modifies all of these deviations from normal both qualitatively and quantitatively.

Inspection of the left ventricular pressure curves shows that the initial pressure is uniformly increased as a result of a leak and that the configuration differs from the normal. The latter involves chiefly changes in amplitude and in the contour of the summits (figs. 1 and 2); in the case of very large leaks the gradients and details of the ascending and descending limbs are changed in addition (fig. 3, B).

Comparison of aortic and ventricular pressure curves and calculations of the durations of successive systolic phases show interesting differences from the normal. As the size of the leak increases, the aortic pressure mounts progressively earlier in relation to the inception of ventricular contraction at *a*; in other words, the isometric contraction phase (*a-b*) is abbreviated. Since this is chiefly determined by the lowered diastolic pressure and only to a minor extent by variation of the ventricular pressure gradient, the degree of shortening is directly related to the size of the leak. The ejection phase (*b-d*) is consistently lengthened, and as the prolongation exceeds the abbreviation of isometric contraction the period of total systole (*a-d*) is also prolonged. Since such effects are a consequence of an increased initial tension in normal hearts it is logical to give a similar explanation in these instances.

The ejection of a normal volume against a lower resistance (i.e., low diastolic pressure) can doubtless produce many of the changes recognized in the aortic pressure curves during aortic insufficiency. When a large artery is suddenly opened and the arterial resistance is thereby reduced the pressure pulse shows similar characteristics; it rises with a steeper gradient and has a larger amplitude. In addition, the isometric period is abbreviated. But in such experiments, the systolic pressure remains definitely below

normal; it never approximates, equals or exceeds systolic pressure as happens within a few beats after production of an aortic leak. Furthermore, the ejection phase is not prolonged. Consequently, the dynamic changes of aortic insufficiency are not entirely accounted for on such a physical principle alone; a physiological reaction capable of increasing promptly the systolic discharge must be brought into operation. Formerly it was supposed that the ventricle empties itself more completely against a lowered pressure; however, the argument itself was fallacious in that such a reaction could not be maintained long unless an added supply were available. Experimental evidence also offers no support for the conclusion. Cardiometer experiments (Wiggers, 1910) showed conclusively that the ventricle does not empty itself more completely during the initial moments after a hemorrhage.

Wiggers (1923) voiced the belief that the restoration of an approximately normal or higher systolic pressure level after aortic insufficiency is due to an increased systolic discharge resulting from an increased diastolic stretch and higher initial tension. An increased diastolic size at constant heart rate was first actually demonstrated by Wiggers, Theisen and Williams (1930). The observations now reported not only establish the existence of an increased initial tension but offer additional evidence for its operation as a compensatory mechanism.

Sometimes the systolic pressure after a lesion does not quite equal the normal pressure previous to a lesion, despite the fact that initial tension elevates and that characteristic prolongation of systole and systolic ejection takes place; but we have reason to believe that in such instances the systolic pressure exceeds the level established according to physical laws alone. The curves of figure 1 are introduced to show such reactions and to direct attention to the fact that the degree of compensation is determined also by the "condition" of the heart and not by initial tension alone.[1]

The height of the initial rise of aortic pressure (*b-b'*) is primarily determined by the duration of the isometric period, but it is also affected by the response of the heart to changes in initial tension. It is therefore rather directly related to the size of the leak but may be accentuated by the increased force of contraction. The steepness of the initial rise of aortic pressure is likewise a resultant of physical laws and physiological reactions. However, these forces are not necessarily additive. Therefore increased gradient of the initial rise (*a-b*) may be associated with a higher systolic pressure as in figure 2, B, or with a lower systolic pressure as in figure 1, B. On the other hand, the gradient may be less when systolic pressure is ele-

[1] These curves should be compared with segments A and B of figure 4 which were obtained from the same animal earlier in the experiment while the heart was in better condition and hence showed a more typical response.

vated as in figure 3, B. These variations are due to the fact that the intraventricular pressure gradient itself is somewhat increased as a result of the increased initial tension in figure 2, B, while it is decreased owing to a more afterloaded manner of contraction in figure 3, B. Similar discrepancies occur as to height and slant of the initial rise. When compensation is good as in figure 2 both the slant and height are increased above normal; when compensation is only fair as in figure 1 the height is affected more than the slope.

The irregular superimposed vibrations are probably caused by the instruments placed in the aorta but the fact that they occur only after the initial rapid rise of aortic pressure supports the previous interpretation (Wiggers, 1928) that the greatest rate of systolic ejection starts after the initial rise at b' and continues until the pressure summit at c is reached.

Having passed the summit c the aortic curves gradually decline until the end of systole at d. A study of many records similar to those shown in figures 1, 2 and 3 indicates that this late systolic decline of pressure (often referred to as the systolic collapse) is greater and steeper when a leak has been produced. Several factors are doubtless concerned, and to varying degrees, viz., the height of the systolic pressure maximum at c, the length of this period (c-d) and alterations in the percentile discharge during early and late systole. As Frank has pointed out, the slope of this portion of the aortic pressure curve is a resultant of the pressure energy developed from moment to moment by ventricular contraction and of the degradation of pressure energy to flow from the arterial branches. The higher the pressure summit, the larger the outflow during a given interval following, and hence the greater the subtraction of pressure energy during that interval. If, in addition, the interval is lengthened, a still lower pressure results at its end. Furthermore, experimental work reported by Wiggers and Katz (1922) indicates that when there is an increase in systolic discharge due to a rise of initial tension, the increment is not proportionately distributed throughout the entire ejection period (b-d); on the contrary, the extra volume is delivered largely by an increase in the velocity of ejection during the maximal ejection phase (b-c). In other words, the added force of ventricular contraction is predominantely exerted during early ejection and is extended to the phase of reduced ejection only slightly if at all. This contributes to the greater systolic decline of pressure with leaks up to considerable size. With extremely large leaks the more rapid decline of the ventricular pressure curve is an additional factor which contributes not only to the steeper late systolic decline but gives the entire summit a more rounded or even a peaked appearance.

Changes in the diastolic portions.[2] A glance at the aortic curves of figures

[2] To avoid confusion the following facts regarding the dynamic changes during ventricular relaxation should be kept in mind: on the records d represents the begin-

1, 2 and 3 shows that the most obvious effect of an aortic leak consists of a more rapid decline of the diastolic portion (after *d*) and that the declivity increases with the size of the leak. Other significant changes are also present that demand more careful analysis. With leaks of moderate size (fig. 1) the incisural fall of pressure (*d-e*) is somewhat steeper and the bottom of the incisural *V* (*e*) occupies a lower pressure level. The postincisural declination (*e-g*) is also more abrupt but a considerable additional fall of pressure still occurs during subsequent diastole (after *g*). Measurements show that the total diastolic fall of pressure is distributed as follows: per cent, incisural decline; 27 per cent, postincisural decline; and 50 per cent, decline during the remainder of diastole. Careful inspection of the aortic curves shows further that the gradient of the postincisural decline is changed at e', i.e., simultaneously with changes in the intraventricular pressure gradient.

The records reproduced in figure 2 illustrate effects which follow the largest leaks produced by a trocar. Comparison of segments 2, B with 1, B shows qualitative differences in the postincisural portions of the aortic pressure curves. The decline (*e-g*) is not only steeper but an additional change in gradient occurs at *f*, i.e., at the moment when ventricular filling from the auricle begins. The decline of pressure during the incisural and postincisural portions of the curve is quantitatively greater, hence by the time ventricular filling begins at *f* aortic pressure has declined to a low level and falls much less after relaxation is complete at *g*.

Measurements indicate that 33 per cent of the total diastolic drop of pressure occurs during the incisural portion (*d-e*), 37 per cent during the postincisural portion and only 30 per cent during the long portion of diastole which follows (after *g*).

It is noteworthy that in both experiments the normal characteristic bends in the descending limb of the intraventricular pressure curves remain unaffected; in each the steep declivity *e-e'* is easily recognizable. In figure 2 the inclinations of the ascending and descending limbs are not affected,

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ning of diastole; *d-e*, the protodiastolic fall of pressure, terminated in the normal heart by closure of the semilunar valves at *e;* isometric relaxation—a phase during which no increase in ventricular capacity occurs—begins at *e* with an abrupt decline of ventricular pressure, but at *e'* this shades into a more gradual slope. Since it has been shown that the a-v valves open approximately at *f* and ventricular filling then commences, the interval *e-f* represents the phase of isometric relaxation while the early portion of ventricular filling occurs during the interval *f-g*. It is significant that such filling is not marked by any sharp inflection of the pressure curve and does not alter its form.

In order to analyze the cardiodynamics of aortic regurgitation succinctly yet accurately it was found necessary to introduce several other terms in our description, viz., *c-d*, systolic decline; *d-e*, incisural decline; *e-g*, postincisural decline; *d-g*, relaxation period.

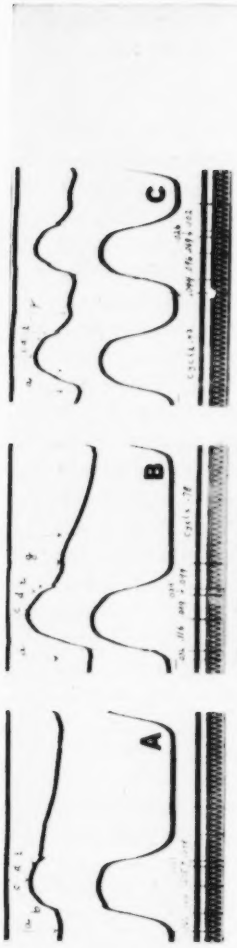

FIGURE 4

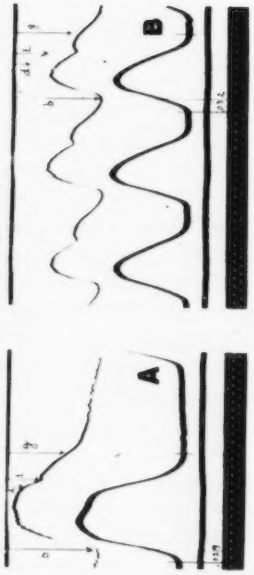

FIGURE 5

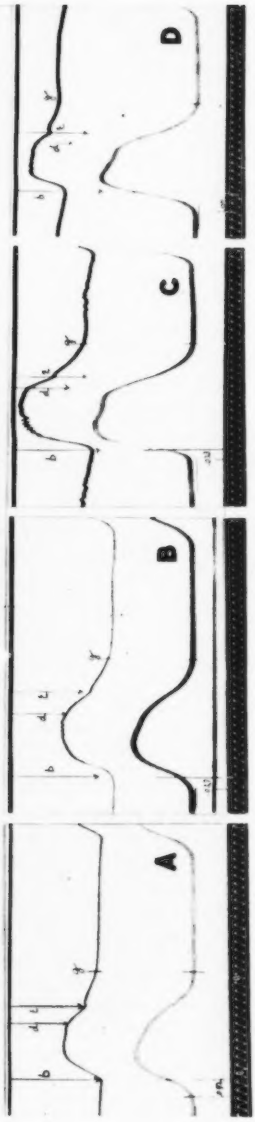

FIGURE 6

while in figure 1 they are slightly less perpendicular. Such differences are not determined by the leak directly; in another record from the same animal (fig. 4) the gradients are slightly steeper. Optical enlargements and reconstructions of many such records have demonstrated that the slope of the curve is only a function of its amplitude; a rise of the pressure maximum as compared to the normal causes a greater steepness; a decrease of the pressure maximum is associated with a more gradual fall.

The curves of figure 3 show typical results when the aortic orifices are kept entirely patent by means of a valve separator. A casual glance shows that the form of the aortic pressure curve approximates that of the intraventricular. But the form of the intraventricular pressure curve is decidedly different from the normal; both ascending and descending limbs are less perpendicular, the top is rounded and the characteristic details in the gradient of the descending limb have vanished; the abrupt fall of pressure which starts at *d* continues uninterruptedly until *e'*. These essential differences between the ventricular curves of figures 2 and 3 are interpreted to signify that in the former the ventricle essentially retains its afterloaded mode of contraction, despite the leak, whereas in the latter it contracts as a loaded muscle. In other words, we no longer have phases of isometric contraction and relaxation when aortic leaks of very large size exist.

These alterations in the mechanism of ventricular contraction and relaxation bring about interesting deviations in the aortic pressure curves. The rise (*b*) which starts nearly simultaneously with the rise in ventricular pressure begins with a gradual curve which quickly changes to an inclination exceeding that of the ventricle. The early diastolic decline of pressure (*d-e'*) is particularly steep and great; it begins abruptly after cessation of contraction at *d* and continues without change in gradient well into the phase of relaxation. In short, no evidence of an incisura at *e* remains. As

Fig. 4. Three segments showing pressure relations in aorta and ventricle. Normal (A), effect of moderate aortic leak at same heart rate (B), and effect of practically doubling heart rate with maintenance of same leak (C). Equivalent lengthened arrows dropped from aortic pressure base line to various points of the curve indicate at a glance pressure changes at several points. Note particularly rise of diastolic pressure, same systolic pressure. Discussion in text (expt. C-491, V).

Fig. 5. Two segments showing effects of extreme ventricular acceleration on pressure curves. Segment A, large leak, heart rate 125 per minute; B, same leak, heart rate 260 per minute. Note in contrast to figure 4, unchanged diastolic and fall of systolic pressure. Discussion in text (expt. C-489, VI).

Fig. 6. Four segments showing effects of lesion and complicating action of increasing peripheral contraction through central vagal stimulation. Segment A, normal; B, effects of large leak; C, same leak with increased arterial resistance; D, same high arterial resistance without leak, immediately after. Change of rate in segments B and C shown not to modify leak. Labels same as in previous record. Note particularly restoration of diastolic pressure to normal levels and further increase in pulse and systolic pressure. Other changes discussed in text (expt. C-489, XII).

a consequence of this rapid early diastolic decline in pressure, 60 per cent of the total diastolic decline precedes ventricular filling at *f* and 87 per cent has taken place by the end of relaxation at *g*.

The differences which occur in the diastolic decline of aortic pressure with leaks of different sizes are partly explained by variations in physical resistance to regurgitation and partly by the altered mode of ventricular relaxation. When the aorta and ventricle are in free communication, their pressures decline with similar gradients during this phase (*d-e*); but when the valves close partially around a trocar or spreader they offer sufficient resistance during the remainder of relaxation to prevent a full and prompt pressure equilization between the aorta and the ventricle. Hence, even with leaks of very considerable size the steep incisural decline of aortic pressure (*d-e*) is abruptly halted at the moment when such approximation occurs (*e*) and the gradient during the post-incisural decline (*e-g*) is far less steep than that in the left ventricle. On the other hand, when valve closure is entirely prevented, the sudden halt corresponding to the incisural *V* disappears completely and the aortic pressure gradient follows that of the ventricle until *e'*. The presence or absence of this incisural halt therefore gives valuable evidence as to whether some degree of closure still persists.

The additional resistance offered by the natural flow of blood from auricle to left ventricle after *f* affects the decline of aortic pressure only when leaks of an optimal size exist (fig. 2). With moderate leaks, the gradient remains unchanged (fig. 1) because the additional resistance caused by blood in the ventricle is relatively small as compared to that due to partially closed valves at the aortic orifice. With larger leaks (fig. 3) the aortic pressure declines so rapidly previous to this that the additional resistance is immaterial.

GENERAL DISCUSSION. We are now confronted with the task of answering some of the questions raised in the introduction of this paper.

A. *Factors limiting the backflow to a fraction of the systolic discharge.* Several factors operate in preventing regurgitation of the entire volume of systolic discharge even under optimal conditions:

By the time diastole begins a considerable portion of the blood volume ejected during systole has been moved to more peripheral portions of the aorta and its branches or has actually flowed out of the arterial system. Consequently, even if the entire volume of the thoracic aortic arch regurgitated it would not be sufficient to fill the left ventricle completely. This limited capacity of the aorta provides an essential factor of safety in limiting the volume of regurgitation even under optimal conditions.

Since the ventricular cavities have a tendency to remain obliterated during the decline of ventricular pressure from *d-f*, space for accommodation of fluid must be forcibly created. Moreover, a greater effective force is necessary to distend the ventricles under contractile stress during the

earlier moments of relaxation than is required after tension has been reduced at the end of relaxation.

An analysis of aortic and ventricular pressure curves has shown that other factors also operate which depend upon the size of the leak. With small and moderate leaks the valvular resistance of partial closure is the significant and dominant factor; with fairly large leaks the additional resistance due to ventricular filling from the auricle comes into play. While these physical factors are absent in the case of extremely large leaks, the backflow is retarded by the physiological reactions of the ventricle, i.e., by the decrease in gradient of ventricular relaxation incurred as soon as the leak is large enough to cause a loaded type of contraction (fig. 3).

B. *The time of maximal regurgitation.* The fact that a diastolic murmur replaces the second sound and that, as a rule, it fades away quickly has generally been regarded as presumptive evidence among clinicians that the chief backflow of blood occurs very early in diastole. A more precise interpretation of successive events by experimental studies made it obvious that if this were true, regurgitation would chiefly precede natural filling of the ventricles from the auricles. Many experimental facts appeared to contradict such a possibility, for instance the fact that no cavity exists within the ventricles and that any regurgitating blood must enter the ventricles against considerable tension. The fact that the descending limb of the ventricular pressure curve shows no change in gradient has also been cited as evidence that no considerable amount of regurgitation could occur previous to auricular inflow. While this observation was again confirmed in these experiments except in case of very large leaks, a reconsideration of the aortic pressure gradients and of the percentile fall of pressure previous to rapid inflow from the auricles makes it necessary to revise our interpretations. Data from the three experiments analyzed and illustrated in this paper show that with leaks of moderately large and extremely large size the decrease in aortic pressure previous to the inflow phase equals 31, 49 and 75 per cent of the total drop during diastole. The decline is obviously increased with the size of the leak and the subsequent decline during the remainder of diastole becomes progressively less until with extremely large leaks it is no greater than might be accounted for by the run off from branches of the aorta.

We cannot escape the conclusions: 1, that whatever the actual volume of backflow, a considerable per cent—which increases with the size of the leak—enters the ventricle previous to opening of the a-v valves, and 2, that the competition for space between blood entering from the auricles and from the aorta postulated by Stewart (1908) is decided in favor of aortic blood because it has the opportunity of getting there first. One of the arguments which has prevented a complete acceptance of the view that considerable backflow occurs is thereby removed.

C. *The discordant features of the aortic and ventricular pressure curves.* The evidence gradually gathered indicates that the regurgitation is of considerable magnitude, but to become firmly convinced that a large volume regurgitates early in diastole the discordant declines of aortic and ventricular pressures must be harmonized. How is it possible for the descending limb of the ventricular curve to remain unaffected with leaks of considerable size while aortic pressure declines much more rapidly than normal? Large numbers of curves were projected, enlarged, redrawn, superimposed and even reconstructed with common ordinate scales, hoping that some obscure deviation from the normal might be discovered. But the only consequence of these labors was the conclusion that as long as the leak is not too large, neither the gradients of the ascending and descending limbs nor the details of the descending limb are affected, except of course inso far as changes in the amplitude of the entire curve affect the gradients slightly.

It gradually became obvious that the fundamental pattern of the ventricular pressure curve is not influenced by entry of blood into the ventricle but is determined solely by its mode of contraction and relaxation, i.e., whether afterloaded as occurs normally, essentially isometric as during severe aortic stenosis, or loaded as in severe aortic insufficiency. The unexpected discovery was made, however, that unless the aortic valves are rather completely incompetent the pattern of an afterloaded contraction and relaxation is retained during aortic regurgitation.

With this fact established the following conception is the only one that explains the discordances between aortic and left ventricular curves and at the same time explains how, despite regurgitation, the normal characteristics are retained with leaks up to those of considerable size: Blood regurgitates during ventricular relaxation because intraventricular pressure falls rapidly, but the mass of blood flowing back and the rate of pressure decline in the aorta are governed also by the resistance at the aortic orifice. The actual increase in ventricular capacity by inflow does not alter the character of the pressure decline regardless of whether this occurs early in relaxation as in aortic insufficiency or late as during natural filling of the normal heart beat.

D. *The relation between size of leak, heart rate, peripheral resistance and magnitude of regurgitation.* It has been questioned repeatedly whether the magnitude of regurgitation is significantly affected when the size of the leak exceeds a reasonable minimum. Allen (1924) found in physical experiments that variations in the aperture from one-half to one-seventh that of the aortic orifice affected the amount of regurgitation very little, while changes in the duration of diastole had considerable influence.

Several observations reported in this paper indicate that the size of leak

plays an important rôle in determining the magnitude of regurgitation.[3] In the first place, under controlled conditions of heart rate, the diastolic pressure decreases progressively when the valves are spread apart farther and farther, suggesting a direct increase in regurgitation up to the maximum size of orifice. Secondly, the decline of aortic pressure previous to ventricular filling from the auricle becomes more and more abrupt as the size of the leak is enlarged. This is of importance because as the percentile regurgitation during isometric relaxation increases there is less competition for space with blood entering from the auricle and hence the total regurgitation is apt to be greater.

The few data of table 1 illustrate the actual and percentile reduction of diastolic pressure found in one experiment (C-521, III, IV, V), two segments of which are reproduced in figure 3.

Our results do not favor the generally accepted view that an increase in heart rate with consequent abbreviation of diastole is capable of affecting the degree of regurgitation significantly, if at all. This is chiefly accounted

TABLE 1

	INCISURAL DECLINE		POST-INCISURAL DECLINE		DIASTOLIC DECLINE	
	mm. Hg	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*
Normal	18*	33	0*	0	36	67
Mild insufficiency	42	42	6	6	48	48
Extreme insufficiency	132	66	18	9	48	25

* Temporary decline followed by subsequent rise.

for by our observation that the greatest regurgitation occurs early in relaxation and that no significant reflux takes place during the portion automatically cut off from diastole by any degree of acceleration likely to take place. This is illustrated by the three segments of figure 4, in segments B and C of which the pressure changes with the same leak but with heart rates of 77 and 140 per minute, respectively, are shown. We note that approximate doubling of the heart rate scarcely affects the systolic pressure maximum (*c*), that the pressure at the end of systole (*d*) is a trifle higher owing to an abbreviation of the ejection phase, and that the pressure level at the incisura (*e*) is correspondingly raised; but the post-incisural decline of pressure (to *g*) is correspondingly steeper with the result that the pressure at *g* is a trifle lower at rapid heart rates.

The abbreviation of diastole at faster rates prevents diastolic pressure

[3] It may be added that failure of previous experiments to demonstrate crucially the occurrence of an appreciable backflow may in part be attributed to their failure to produce leaks of sufficient size. We are convinced that cutting or tearing of valve leaflets produces only moderate sized leaks.

from falling to so low a level, hence diastolic pressure (measured at *a*) and the pulse pressure tend toward normal values. In other words, cardiac acceleration compensates not by reducing the backflow essentially but by terminating the conversion of pressure energy into flow. With the elevation of diastolic pressure, the isometric period lengthens again, though it never quite approaches normal. Obviously such effects can occur only when the leaks are not too large and when a considerable decline of pressure still occurs after isometric relaxation. When the leaks are large and extremely large as in figures 2 and 3 such an abbreviation of diastole is without any effect as regards the diastolic pressure level.

Reactions of a more complicated sort occur when excessive acceleration supervenes (e.g., as a result of auricular flutter). Figure 5, B illustrates such a condition, the ventricular rate being 280 per minute. We note that now the diastolic pressure remains essentially unchanged but that systolic pressure is reduced and is responsible for the decrease in pulse pressure. These effects clearly are due to the reduced systolic discharge which accom-

TABLE 2

SEGMENT	INCISURAL DECLINE		POST-INCISURAL DECLINE		DIASTOLIC DECLINE	
	mm. Hg	*per cent*	*mm.*	*per cent*	*mm.*	*per cent*
A	36	53	20	29	12	18
B	42	48	36	41	10	11
C	48	40	58	48	14	12
D	22	42	18	35	12	23

panies such a violent change in heart rate. The contour of the ventricular pressure curves reflects the disturbances of contraction; the gradients of both ascending and descending limbs become more gradual and the summits are more peaked. As a result of the slow rise the isometric phase lengthens slightly. Despite the lower arterial pressure at the end of systole (*d*) and the slower gradient of ventricular pressure decline (*d-e*), the incisural decline of aortic pressure (*d-e*) is much greater. The continuance of an abrupt decline during the post-incisural pressure is apparently prevented in this instance by a superimposed wave, presumably of reflected origin.

It is obvious that such changes in the mechanism of contraction materially alter the circulatory dynamics and again demonstrate that conclusions based on simple physical postulates cannot replace animal experimentation.

Inasmuch as peripheral vascular changes, which increase the peripheral resistance to flow and which in themselves tend to produce hypertension, are frequently associated with regurgitation clinically it appeared advisable to study the influence of such changes experimentally. This was done by compressing the aorta mechanically or by inducing a general reflex vaso-

constriction by stimulating the central end of the vagus nerve. The effects were similar and are illustrated by the records of figure 6, B and C. Comparison of the pressure alterations shows that after vasoconstriction (segment C), the initial intraventricular tension is increased and the intraventricular pressure gradients are steeper. The aortic pressure curves indicate that the systolic pressure is elevated considerably and that diastolic pressure returns to normal levels illustrated in A, but the pulse pressure is increased further. The rise of pressure is steeper and the pressure at the end of systole is also greater. The interval of isometric contraction is not essentially changed in length, the effect of a higher diastolic pressure being nicely counterbalanced by the increased velocity of the ventricular pressure rise.

In evaluating the effect of hypertension it is important to keep in mind the changes both in the actual volume regurgitating and in the percentile regurgitation, for the latter has a bearing on the net discharge. The calculations incorporated in table 2 confirm impressions derived from a direct inspection of the records. It is noted that in hypertension the incisural fall of pressure (2nd column) is actually greater, but the percentile decline (3rd column) is less. Both the actual and the percentile post-incisural fall of pressure (4th and 5th columns) are increased, and increased to such an extent that the total early diastolic pressure drop is greater at the higher pressures. This undoubtedly signifies increased diastolic backflow. However, the increase in percentile regurgitation during the post-incisural phase is offset by the decrease during the incisural phase so that the percentile regurgitation for the entire cycle remains practically unchanged.

SUMMARY

In order to evaluate the pressure changes produced by the operation of purely physical laws and by simultaneous alterations in the physiological responses of the heart, and in order to harmonize seeming discrepancies in results obtained by pressure and volumetric studies of aortic regurgitation, the effects of experimental aortic leaks on the pressure changes in the aorta and left ventricle were reinvestigated.

For this purpose optical pressure curves were recorded by our usual technique from dogs whose heart rate was slow, constant and under control. Leaks of different sizes were produced in some cases by an instrument previously described and in others by a newly designed valve spreader which enabled us to produce maximum leaks uncomplicated by stenosis.

Analysis of the systolic portions of the pressure curves elucidated two points, viz.: *a*, the causes of the greater steepness, larger amplitude and high systolic pressure following leaks, and *b*, the steep decline of pressure late in systole, i.e., the true "systolic collapse." The former characteristic phenomena were found to be due mostly to the lower arterial resistance

against which ejection begins in aortic insufficiency, but the increased systolic discharge which promptly occurs as a result of an increased initial tension contributes materially to these changes and is solely responsible for the restoration of systolic pressure to or above normal levels. The redistribution of the percentile discharge so that the increment of ejection is expelled largely during the early phase of ejection fully accounts for the steeper systolic decline following the pressure summit; but it should be noted that the intensification of this systolic decline (i.e., the so-called "systolic collapse") has been greatly over-emphasized.

The increased diastolic decline of pressure often referred to as the "diastolic collapse" was carefully studied as regards the magnitude and steepness of the pressure drop which precedes and follows ventricular filling from the left auricle. It was found that with even moderate leaks 40 to 50 per cent of the pressure fall may occur during isometric relaxation, while with maximum leaks this may equal 80 per cent of the total decline of pressure during diastole. In all but maximum sized leaks this early diastolic decline of aortic pressure occurs in two stages designated as incisural and post-incisural which are clearly separated by a halt and change of pressure gradient. With full sized leaks a continuous abrupt decline of pressure takes place.

The evidence is conclusive that whatever the actual volume of backflow (which must be established by other types of experiments) a considerable per cent, increasing with the size of leak, flows back previous to the opening of the a-v valves. Thus the postulated competition for space between aortic and auricular blood does not exist to an essential degree. One of the arguments which has prevented a complete acceptance of the view that considerable backflow occurs is thereby removed.

A second difficulty, viz., that the gradient of the ventricular pressure decline is unaffected by a leak, now appears explainable. In corroboration of the previous observations of Wiggers (1915) it was found in more completely controlled experiments that the ventricular relaxation gradient remains unaffected unless leaks of extreme size are produced, in which case the decline becomes more gradual. We concluded upon the basis of many curves that the ventricular relaxation gradient is not determined nor affected by entry of blood into the ventricle, but like the contraction gradient is regulated solely by the mode of ventricular contraction, i.e., whether loaded or afterloaded. The unexpected and important discovery was made, however, that unless practically complete incompetence of the aortic valves exists, the ventricle continues to contract according to an afterloaded pattern.

In evaluating the effect of secondary factors on regurgitation the effects both on actual and percentile regurgitation must be considered, for the volume of ventricular discharge usually changes. Only by so doing can

their beneficial or harmful effects as regards total and net discharge be evaluated. It was established that the size of the leak is the chief factor which determines both the actual and percentile decline of pressure during isometric relaxation and hence, contrary to previous opinion, is the chief factor which modifies the degree of regurgitation. On the other hand, it was found that such changes in heart rate as are likely to occur in patients affect the pressure drop relatively little in case of small leaks and not at all when the leaks are large. Finally, it was demonstrated that although the actual volume of regurgitation is definitely increased by augmenting the peripheral resistance the percentile regurgitation is not altered as long as the ventricle is capable of responding with an increased stroke through a further rise of initial tension. Thus while the external work is increased by a concomitant high resistance the net discharge remains unaffected.

Since many of these effects are contrary to results obtained from artificial physical schemes it again impresses the fact that conclusions so derived cannot replace those yielded by animal experimentation.

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STUDIES ON THE UTERUS

V. The Influence of the Ovary on the Motility of the Non-Gravid Uterus of the Unanesthetized Rabbit[1,2]

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In a previous study we have called attention to the fact that prior to coitus, unanesthetized female rabbits usually exhibit a marked or a moderate motility of the uterine fistula (Reynolds and Friedman, 1930a). Earlier we called attention to the activity of uterine fistulae of four normal rabbits, over extended periods of time (Reynolds, 1930a). Two of these rabbits had, at one time or another, uteri which were completely quiescent, whereas at other periods there was marked activity which was in striking contrast to the inactive state. In view of these results we were tempted to suggest that a marked type of uterine activity might possibly be associated with heat, and the quiescent condition, with the absence of heat. Although there are now in the literature data obtained on the excised uterus which would seem to support such a view, nevertheless we do not consider that such data were strictly comparable to the results one may obtain in the intact animal. For example, we have shown (Reynolds, 1930b) that the quiescent uterine fistula does not respond to the intravenous injection of pituitary extracts, whereas the excised uterine strip never fails to respond to such preparations. Because of such a discrepancy between the results obtained in the *in vitro* and in the *in vivo* experiments, it seemed advisable to investigate in the intact, non-pregnant animal, the rôle of the ovary in regulating uterine motility. This we have done by determining:

1. The uterine motility associated with oestrus and anoestrus.
2. The uterine motility in the castrate female, and so in the absence of all influence of the internal secretions of the ovary.
3. The response of the uterine fistula of the castrate to the injection of lipoid-soluble and water-soluble oestrogenic extracts.

[1] The expenses of this research have been aided by a grant to Prof. H. C. Bazett from the Committee for Problems on Research of Sex of the National Research Council. The funds were administered by Dr. M. H. Friedman.

[2] The work of this paper has been accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in this University.

[3] George Lieb Harrison Fellow in Medical Sciences, 1930–31.

The operative technic and recording procedure which we have developed for recording uterine motility in the unanesthetized chronic rabbit have been described elsewhere (Reynolds, 1930a; Reynolds and Friedman, 1930a), and will not be considered here except as it appears necessary in describing the modifications that were made in the examination of the several points under investigation.

EXPERIMENTAL RESULTS. 1. *Uterine activity associated with oestrus and anoestrus.* The rabbits used for this phase of the work were taken out of our general stock, the care of which we have already described (Reynolds and Friedman, 1930a). It was known, by segregation of the does or by laparotomy, that the ovaries of these animals contained no corpora lutea at the beginning of each experiment, and through the course of each experiment the rabbits were isolated from each other and from the bucks, except at the times when matings were attempted. Fistulae were made, and the animals allowed to recover for four days in one instance, to several weeks in others before an attempt to mate them was made. In every instance a control record of uterine activity was obtained, after which the doe was placed with the buck. Whether or not coitus occurred, was determined by observation and by examination of the vagina for sperm. The ovaries of those does which accepted the male were examined at the end of a period of pseudopregnancy for corpora lutea, as evidence of ovulation.

A summary of the observations which we have made on the correlation of uterine motility with heat or the absence of heat are briefly tabulated in table 1. Here it may be seen that twenty-six does accepted the male, or were known to be in heat (immediately post partum; Hammond and Marshall, 1925). Sixteen of these showed rhythmic, marked activity; eight, moderate activity, and two, feeble activity. Of does not in heat, fourteen refused the male 23 times while showing feeble activity; seven refused the male 12 times while showing moderate activity. No does that showed marked activity ever refused the male.

Of the seventeen does which at one time or another refused the male, three did accept the male later, and so had come on heat. One of these showed no change in the moderate activity of her uterus from July 19th until August 23rd, at which time she had coitus. Another, on the other hand, showed within the space of one week, activity increasing from a feeble irregular sort, to a slow rhythmical, marked type of activity when she accepted the male. Still a third one had coitus when she was exhibiting a feeble, irregular sort of activity. These three animals present several distinct and contrasting histories of uterine activity before coitus, and are not, in the main, in accord with the larger part of our results cited above. A consideration of them, and of the last one in particular, will be deferred until later.

As regards a correlation of the frequency of the occurrence of the uterine contractions, and the presence or absence of heat, there appears to be no regular finding. The intervals between the commencement of the contractions of moderate size in the anoestrus animals have ranged from 20 to 90 seconds; in oestrus animals, from 20 to 60 seconds. Of those animals in oestrus which showed marked activity, the range of the frequencies is more or less 60 to 90 seconds, although one of the does showing the most marked activity was one whose uterus contracted once each 30 seconds. Possibly the most definite correlation apparent from our results is that feeble contractions, as recorded by our method, are quite irregular, and are most

TABLE 1

Summary of data correlating uterine motility with oestrus and anoestrus

Types of activity may be designated in the following manner:

0 = Quiescent condition of uterus in which no activity can be recorded with our system.

\+ = Feeble activity is that which with our system of recording gives records of contractions of ½ inch or slightly less.

++ = Moderate activity is that which varies between ½ inch to 2 inches, but which appears to average 1 inch to 1½ inches.

+++ = Marked activity is that which varies between 2 inches to 4 inches.

++++ = Marked activity, over 4 inches.

In all these classes of activity one may find rhythmical (regular) activity, or arhythmical (irregular) activity. Frequently with feeble activity one sees a sort of undulating movement, constant in rate on any one animal for a short period of time, but varying in rapidity from animal to animal.

TYPE OF ACTIVITY	ANOESTRUS		OESTRUS	
	Number of does	Number of instances	Number of does	Number of instances
0 or +	14	23	2	2
++	7	13	8	8
+++ or ++++	0	0	16	16
Total.....	21	36	26	26

characteristic of anoestrus, while in contrast to this, the activity of does in heat is almost always rhythmical.

2. *Uterine motility in the castrate rabbit.* A brief summary of the data which we have obtained on spontaneous uterine motility following complete ovariectomy is presented in table 2. These data are taken from experiments done in connection with the latter part of this paper. With the exception of the rabbits used in the experiments with the lipoid-soluble Estrogen, we have used only does which have dropped a litter one to fourteen days before castration. By so doing, all the uteri were in the oestral stage at the time of castration. Two series of experiments were performed,

one on "recent" castrates, and the other on "old" castrates. In the former series (26 animals) records were obtained three to six days after castration, in the latter (11 animals) no records were made until three weeks after ovariectomy. In eight rabbits, included in the first series, records of uterine activity were obtained while the does were in heat before castration as well as for three or more days after ovariectomy.

The motility of the uterus, both early and late after castration, may be seen in table 2. The characteristic changes which regularly follow removal of both ovaries may be listed as follows: 1, all rhythmicity is soon lost; 2, a majority of the rabbits exhibit a quiescent uterus or one which shows only feeble irregular activity; 3, these effects on rhythmicity and amplitude of the contractions are pronounced, if not complete, within two to four days following castration; 4, at the expiration of three weeks the uterus is completely quiescent (ten of eleven cases).

TABLE 2

Summary of data correlating uterine motility with absence of ovarian influence: ovariectomy

TYPE OF ACTIVITY (IRREGULAR)	RECENT CASTRATES		OLD CASTRATES	
	Number of does	Number of days castrated	Number of does	Number of days castrated
0	14	3–10	10	20–21
+	7	2–6	1	21
++	5	2–5	0	
+++	0		0	
Total.....	26		11	

The characteristics just described are derived chiefly from isolated observations on rabbits after castration has been performed, with no control runs obtained while the ovaries were yet intact. But the experiments on the eight rabbits in which records were obtained both before and after castration demonstrate that the loss of all but feeble irregular activity must be ascribed to the absence of the ovaries. In these experiments we recorded the motility of the uterus immediately *post partum*, while one or both ovaries were still intact, and again on subsequent days after partial or complete ovariectomy. One to four days *post partum* one ovary was removed and fistulae made, in four rabbits. Uterine activity was recorded on subsequent days, until moderate rhythmic contractions were seen, or until there was no increase in the amplitude of the contractions. Recovery from the effects of the operation seemed to be achieved in the course of two to three days (see fig. 1). The second ovary was removed on the fourth day after the first operation. The pro-

found effect that set in as a result of this second operation, or complete castration, in every instance affecting both the amplitude and rhythm of the contractions, is obvious from the records we have obtained. The decrease in activity persisted until the sixth day of castration at which time these four rabbits were injected with Theelin as described below. Four rabbits were used as controls, in that, at the time the fistula was prepared, both ovaries were left intact. Activity was recorded on the

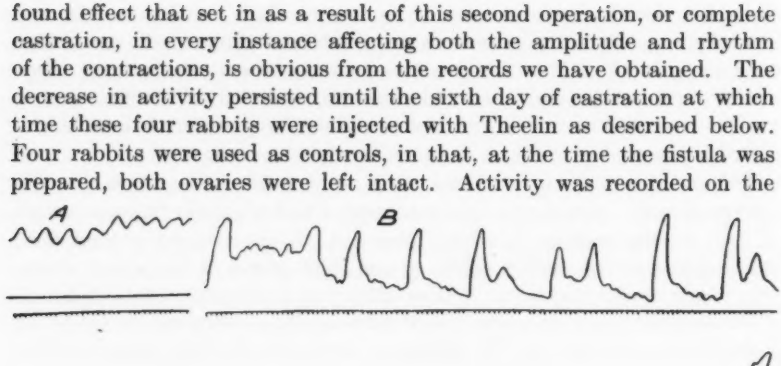

Fig. 1. Rabbit 41. Records showing the motility of the uterus two days after preparation of the fistula, *A*; a return to the oestral type of activity on the third day, *B*; the effect of ovariectomy 2, 4, and 6 days after castration, *C*, *D*, *E* respectively; and 24 hours after replacement therapy with 2 r. u. oestrin per kilogram body-weight, *F*. Protocol of this experiment:

	2/12/31	Litter born; removed immediately.
	2/13/31	Preparation of fistula. Left ovary removed at this time.
A	2/15/31	Activity of uterus two days after operation.
B	2/16/31	Activity of uterus three days after operation. Complete castration by removal of right ovary through flank incision.
C	2/18/31	Activity two days after complete castration.
D	2/20/31	Activity four days after castration.
E	2/22/31	Activity six days after castration. Weight of rabbit, 2.5 kgm. Four intravenous injections of 1.25 r. u. (0.025 cc.) Theelin each at 2½ hour intervals, (total 5 r. u., or 2 r. u./kilo).
F	2/23/31	Record showing return to activity 24 hours after first injection of Theelin. Rabbit killed at once.

Autopsy findings of uterus: good condition; free from all inflammation, infection, perforations and adhesions. Some "uterine milk." Sites of ovariectomies, free from infection; with only slight adhesions.

M, mechanical responses elicited by pressure on abdomen to indicate that the balloon is freely compressible.

One-fifth original size.

second and third days subsequently, and on this latter day, one ovary was removed. No pronounced or persistent effect of the operation was seen. The second ovary of each of these animals was later removed, and the typical castration syndrome developed promptly, to a profound extent. Two does were not castrated but were maintained as controls. They showed persistence of moderate activity in one instance, marked activity in the other, and both showed a willingness to copulate when placed with the male. These results conclusively show that the changes which we have described that are a consequence of castration of a doe in heat are due primarily, if not entirely, to the loss of the immediate influence of the ovaries.

Attention is called to the fact that of all the recent castrates, only one showed a moderate, regular activity; it is the *only* one of our recent castrates that has ever spontaneously accepted the male. This was on the second day following castration during oestrus. Four and six days after castration, the activity of her uterus was both feeble and irregular,—a type of activity which is in agreement with the data obtained from the other seven rabbits of this group of experiments. In all eight, then, we have demonstrated that marked or moderate activity of oestrus becomes, as the result of ovariectomy, progressively less and less, and is quite irregular within the space of two to four days. There is likewise a loss of heat concurrently with these changes. These findings are in agreement with the nineteen isolated observations on our other castrates, to which reference was made above.

3. *Substitution therapy with oestrus-producing substances.* The oestrus-producing substances which we have used are the water-soluble Theelin, and the lipoid-soluble Estrogen. These substances were supplied in ampoules, the former stated to contain 50 rat units, the latter, 25 rat units per cubic centimeter of solution. Estrogen is obtained in the lipoid phase resulting from suitable extraction of human placentae. Theelin is a crystalline substance, dissolved in water. It is extracted from human urine of pregnancy by the method of Veler, Thayer and Doisy (1930), and is prepared and supplied to us by the Department of Experimental Medicine, of Parke, Davis and Co. Both of these preparations have all of the oestrogenic properties ascribed to follicular fluid by many investigators in recent years. Allen and Doisy (1927) have reviewed the properties of such substances, as have Parkes (1929), and Frank (1929) more recently.

a. *Water-soluble oestrin: Theelin.* The rabbits which we have used in the Theelin series of experiments have been mentioned above. They were castrated one to fourteen days *post partum.* Normal, or control records were obtained about the fourth day subsequently, the Theelin injections were commenced immediately thereafter, when little or no

spontaneous activity was present. The dosage was administered in the desired number of rat units per kilogram body-weight. To obviate variable factors in the rate of absorption and inflammation at the site of injection, the intravenous route was used, and to retard wasteful elimination as much as possible, we have made four injections at two and one-half hour intervals, over a period of about eight hours in all. This has been an arbitrary and routine procedure to standardize the conditions as much as possible. In order that it might not be argued that we were using excessive quantities of Theelin, we have worked for minimal effects rather than maximal. We regard as important the fact that in no instance, in the recent castrates, has the sum-total of the four injections in any one doe exceeded one cubic centimeter of solution, or a fourth of a cubic centimeter per injection, and positive results have been obtained with as little as four injections of 0.006 cc. each. The single mass injection, at the termination of each experiment, however, did exceed one cubic centimeter of solution. When the smallest quantities of Theelin were used, it was measured accurately with a tuberculin syringe, and diluted with water. In no instance did the four injections of the dilution exceed 0.5 to 1.0 cc.

The general character of the response in these animals was most consistent throughout. In some instances there was the beginning of an increase in activity within six to ten hours which was at best only slight. By fifteen to twenty hours, considerably greater increase occurred. In all cases the maximum of activity was seen about twenty-four hours, or a little later, after the first injection of Theelin. Maximal activity continued in some instances until the second day, in others the peak of activity was over, and a subsidence of activity commenced. This decrease in activity proceeded at different rates in the several animals during the course of three to six or more days. When the activity had subsided until it resembled that seen before the injections, a second injection was made in most instances, to demonstrate that the uterus was still capable of a response. The second injection was a mass dose of usually 100 r. u. intravenously, and 100–200 r. u. subcutaneously. Records were obtained only once after such an injection, usually a day later, and just before the rabbit was killed and the uterus grossly examined for inflammation or infection. In our autopsy findings we have found local mucosal involvement in three instances. There is no reason to suspect, in these three cases, that the activity was modified by these pathological areas, inasmuch as infection in non-gravid uteri is, in our experience, accompanied by exaggeration of motility rather than a diminution. In none of these instances were the results anomalous, or different from those obtained in other rabbits whose uteri were found to be entirely normal in gross appearance.

Attention should be called to three exceptions (not those showing mucosal involvement) among our recent castrates which are possibly ex-

plicable on the basis of some variation in the value of the threshold dose. In two animals a total dose of 0.5 r. u. per kilo failed to give a response, even though one other animal in apparently a similar condition did give a response to this amount. It seems likely, therefore, that 0.5 r. u. per kilogram body-weight is sub-liminal for a definite response, although this cannot be definitely stated on so small a number of experiments. Two rat units per kilo regularly gave a response, however, and this amount is certainly above the threshold for a uterine response to Theelin. In yet another animal, the third exception, injected on the fourth day *post partum*, a dose of 5 r. u. per kilo gave no response, though two others at a similar period after parturition did respond to 15 r. u. per kilo. It is barely possible that some persistent effect from the recent pregnancy may still exert an influence on the first few days *post partum*, giving rise to a differential threshold to Theelin yet which is inoperative on the sixth day or

TABLE 3

Summary of data showing increase in uterine motility resulting from substitution therapy (Theelin): change in 24 hours

RECENT CASTRATES						OLD CASTRATES				
Increase in activity (irregular)	15 r.u. per kilo	5 r.u. per kilo	2 r.u. per kilo	0.5 r.u. per kilo	Total rabbits	Increase in activity	30 r.u. per kilo	20 r.u. per kilo	15 r.u. per kilo	Total rabbits
No change	0	1	0	2	3	No change	0	0	3	3
0 → +	0	1	0	0	1	0 → +	2	1	0	3
0, + → ++	0	2	2	1	5	0, + → ++	2	1	0	3
0, + → +++	2	2	3	0	7	0, + → +++	0	1	0	1
Total.....	2	6	5	3	16		4	3	3	10

later, when other recently castrated does responded with as little as 2 r. u. per kilo.

In general, our results on the old castrates are like those described for the recent castrates. The most apparent difference is not in latency,—which remains surprisingly constant,—but in the minimal effective dose required to elicit a response. We have obtained consistent positive responses in recent castrates with 2 r. u. per kilo, and in one instance with 0.5 r. u. per kilo. If we assume 2 rat units per kilogram body weight to be about the minimal effective dose, that for old castrates is found to be approximately ten times this amount, or between 15 and 20 r. u. per kilo. Three does gave negative results with 15 r. u. per kilo, whereas three others gave definite responses with 20 r. u. The only noticeable effect obtained with the smaller amount, 15 r. u., is that in one instance an irregular type of activity became regular within twenty-four hours, and a

good response was obtained after the mass injection which was made on the second day. A summary of the experiments indicating changes in motility as a result of Theelin administration is given in table 3.

b. *Lipoid-soluble oestrin: Estrogen.* Our experiments with Estrogen were not conducted with as rigorous a routine as were the Theelin experiments. Rabbits were used indiscriminately from our stock, irrespective of recent pregnancies. Single intravenous injections were made, and the number of days between castration and the day of injection was in most cases a matter of chance. Nevertheless, in spite of these irregularities in the routine, chief among these being the utter disregard of oestrus and anoestrus at the time of castration, the results are qualitatively alike among themselves, and are strikingly comparable in every way to the results which we have obtained with Theelin. In table 4 are listed three castrates which gave no response, whereas fourteen others did, with a small dosage in rat units per kilogram body-weight. These results,

TABLE 4

Summary of data showing increase in uterine motility resulting from substitution therapy (Estrogen), irrespective of dosage

CHANGE IN ACTIVITY	CASTRATED 2 TO 5 DAYS	CASTRATED 9 TO 16 DAYS	TOTAL RABBITS
No change	2	1	3
0 → +	0	0	0
0, + → ++	3	6	9
0, + → +++	2	3	5
Total.....	7	10	17

obtained with what is considered a relatively crude extract which was not given under uniform conditions, is added evidence that it is the oestrus-producing substance or substances of placentae or urine of pregnancy that are effective in producing uterine activity in our castrate rabbits.

DISCUSSION. *Oestrus and anoestrus.* If functional changes in the ovary exert a regulating influence on the motility of the uterus, along with controlling some of the known phenomena which are characteristic of sex behaviour in the female rabbit, then there are four conditions with which one should be able to correlate uterine motility. First, it is highly probable that during pregnancy the uterus is quiescent until the time of parturition, else abortion might occur spontaneously. The work of Knaus (1927) would support such a view, notwithstanding evidence to the contrary reported by Clark, Knaus and Parkes (1925–6). During a second condition in the rabbit, pseudopregnancy, the uterus is known to be quiescent until the termination of this period when uterine motility returns (Reynolds and Friedman, 1930a), and the doe comes again into heat.

A third condition of ovarian activity which might affect uterine motility is that of oestrus, and lastly, the condition of anoestrus might be expected to affect uterine motility in an opposite manner.

The periods at which female rabbits will copulate are varied and uncertain. Moreover, they will frequently accept the male under the most dissimilar conditions, and it is for this reason that we must arrive at a definition of the oestral condition, although it may be subject to change in the light of new work. Ordinarily, when a mature female rabbit copulates, a series of changes takes place within the animal which lead to ovulation in the course of ten or more hours. Sometimes, however, a non-pregnant female fails to ovulate in response to the stimulus of coitus; especially is this so in young does copulating for the first time. Pregnant or pseudopregnant does will accept the male sometimes, although ovulation, in the rabbit, is not known to occur at this time. The question arises, therefore, just which of these conditions may be considered oestral. Is the mere willingness of a doe to copulate indicative of oestrus, or is the willingness to copulate, plus the ability to ovulate, a more satisfactory definition of oestrus? The latter view is the one that appeals to us, and is the sense in which we have used the term "oestrus" in this work. Conversely, we regard anoestrus as a condition of unwillingness to mate in sexually mature non-pregnant, non-pseudopregnant rabbits which have functional ovaries. We realize that these definitions may not account for all the conditions one may see in the rabbit. For example, it is difficult to accept the condition we observed in a castrate doe as oestral, although this animal, on the second day of castration showed a moderate, regular activity of her uterine fistula, and exhibited all the accepted external signs of oestrus. One cannot say that she was not in heat, for she accepted the male four times in a few minutes, yet for our purposes it is impossible to consider that the condition of desire which she exhibited was necessarily comparable to oestrus in a normal, intact animal.

With the limitations of the definition stated above, it may be seen from our results, that in no case where marked uterine motility was seen was that doe not in oestrus. On the other hand, of twenty-three instances of does in anoestrus, seventeen of these showed only feeble and usually irregular activity, or else none at all. We are confronted with some irregularities, however, if indeed uterine motility *is* correlated with oestrus as we have defined it. One may see, in table 1, that of twenty one instances in which moderate activity was seen, approximately a third of these occurred during oestrus, the remaining two-thirds in anoestrus. Also, two does accepted the male while showing only feeble activity. Such irregularities are not easily explained. In our experience with mating rabbits we have frequently noted that of does which eventually accept the male, some copulate much more willingly than do others. Thus, in some

few cases, does may have coitus just once after many attempts on the part of the male. All subsequent attempts by the same buck or others are refused. More often, however, does will repeatedly accept the male, each time exhibiting marked lowering of the back, accompanied by elevation of the hind quarters and the tail reflex described by Parkes (1930a). It is indeed unfortunate that at the time when these apparent anomalies in our results presented themselves, no cognizance was taken of the eagerness, or lack of it, with which these does accepted the male. It is our impression, however, that when marked uterine activity is seen, the doe always shows great willingness to copulate. This is especially true of *post partum* does. It is barely possible, then, that some accounting for the irregular results may be made on this basis. Moreover, it must be emphasized that although our classification of activity is entirely arbitrary, any further subdivision does not clarify the situation at hand but, rather, complicates it. Nevertheless, despite the irregularities we have noted, we feel, in the light of the records we have seen, that the ovary is a factor of prime importance in regulating the motility of the non-gravid uterus of the rabbit, particularly in view of the striking correlation which exists between marked uterine activity and oestrus, and between little or no activity and anoestrus. Parkes points out (1929) that a general conclusion which may be drawn from other work on the excised uterus, is that feeble, rapid contractions are characteristic of dioestrus, while fewer but more powerful ones occur in oestrus. The work of Blair (1923), Keye (1923), Frank, Bonham and Gustavson (1925), and of Wislocki and Guttmacher (1924) support such a view. Clark, Knaus and Parkes (1925) however, find this generally to be the case, but state that no "certain" relationship between the stage of oestrus and the frequency of contractions exists, when records were made on the uterus of the anesthetized mouse and on the same uterus excised. Because of the irregularities in oestral activity which we have seen, however, we are obliged to add that there may be times, though rarely, when extraneous influences, which are at present unknown, modify or annul the effect of the ovary in the influence which it exerts on uterine motility. They may be nutritional, pathological, or operative, and in any event probably reside outside the ovary, since the rabbits showed other accepted signs of oestrus.

Castration. A still further line of evidence which supports the thesis that the ovary is of prime importance in regulating uterine motility, is to be found in the thirty-seven castration experiments reported above. We have seen that the complete absence of the immediate influence of the internal secretions of the ovary is associated with a partial loss at first, and a complete loss eventually, of all uterine motility. This occurs without exception, and as surely as atrophy of the uterus follows complete ovariectomy (Hammond and Marshall, 1925), although the loss of motility

appears to take place far more rapidly than does atrophy, and not *pari passu* with it. The experiments which we have performed on eight rabbits in which records of activity were obtained before castration, and on the days after it, leave no doubt that the effect may be attributed solely to the loss of ovarian influence.

Our results on the uterus *in vivo* lend support to the current idea, gained by studies with the uterus *in vitro*, that the uterus of the ovariectomized animal is quiescent. These effects have long been studied on the excised uterine strip. Athias (1919–20) reviews in detail the older literature bearing on this point, so that it need not be cited here. It will suffice to say that by the data obtainable by means of such technic, small undulating contractions are generally believed to characterize uterine movements in castrate animals. The results obtained by our method, and especially in those cases where records were obtained before and after castration, are in general agreement with the data obtained with other technics.

Replacement therapy. We regard the uniformity of the uterine response of the castrate to the injection of Theelin and Estrogen as being so regular and reproducible, that there can be little doubt but that the effects which we have observed are due to a potent substance in the oestrogenic extracts which we have used, and not to some non-specific effect. Moreover, it is our experience that saline, oxytocic substances or other extracts never induce activity in quiescent uteri of unanesthetized rabbits. Pituitrin, pitocin, gynergen (ergotamine tartrate) and histamine (0.025–0.01 mgm.) are impotent on all quiescent uteri of unanesthetized rabbits where we have tried them. Furthermore, in work soon to be reported (Weinstein, Reynolds and Friedman, 1931), we have been unable to produce an anaphylactic response in sensitized quiescent uteri, although a response can readily be obtained in the sensitized active uterus. It is, therefore, no doubt significant that, in our experience, Theelin and Estrogen are the *only* agents by which activity in inactive uteri has ever been induced.

We believe that the long latency which we have invariably obtained presents one of the more important aspects of these results with oestrin on the uterus *in vivo*. There is, however, some recent work which purports to show that oestrin has an *immediate* effect upon the uterus *in vitro*. We do not deny that this is so, but we do doubt the physiological significance of it. In our work we have seen that a full half-day elapses before there is any appreciable effect to be noted in the response to liminal amounts of Theelin, and it is twenty-four hours or more before the peak of activity is over. A latency of this duration is not unknown in other humoral mechanisms in the rabbit. Ovulation, which depends upon a humoral mechanism (Friedman, 1929), requires over ten hours. Our results, therefore, are in striking contrast to those of Brouha and Simonnet (1927a; 1927b) who report that a contracting uterus from an animal in heat becomes quiescent

by merely washing it for twenty minutes with an oestrin-free perfusion fluid, and that the addition of oestrin to any quiescent uterus promptly initiates activity. Bourne and Burn (1928) however, find that only "especially sensitive" uteri *in vitro* will respond to oestrin, and Fraenkel (1927) reports completely negative results. Durrant (1930), moreover, finds that commercial oestrogenic extracts diminish or even abolish activity of the uterus *in vitro*. Because of these contradictions, and because of the immediate effect of oestrin on the excised uterus, one wonders as to their significance, especially in the light of the excellent work of Van Dyke and Hastings (1928) who have shown the profound influence of ionic imbalance on the motility of the excised uterus, particularly of the calcium ion. From the work of the latter investigators, it appears that results obtained in response to the addition of drugs and extracts to the fluid which bathes an excised uterine strip cannot have real physiological significance unless the ionic environment of the tissue be quantitatively controlled, both before and during the experiment. Our work, therefore, would appear to substantiate the work of some of these other investigators, but we believe that because we have demonstrated, on one and the same uterus *in vivo*, the response of the oestrogenic extract we have used, that our results present a physiological picture that may not be justifiably inferred from theirs, and hence the agreement is more apparent than real.

The finding that oestrin will produce activity in the quiescent uterus appears to present a paradox, namely, that this substance which produces and sometimes regulates uterine contractions is obtained from human urine of pregnancy, yet it does not produce abortion in the woman from whom it was obtained. We must suppose therefore, that its effect on the human uterus is of so little consequence that it will not terminate pregnancy, or else that it is directly or indirectly rendered ineffective. There is some evidence that the latter view may be correct. One of the properties of Theelin is that its effect even in minute quantities, is prolonged over many hours, or even days. If then, we may assume that Theelin is identical with the oestrus hormone or hormones of the rabbit, we must admit that the rapid cessation of uterine motility that occurs within a few hours after coitus (Reynolds and Friedman, 1930a) is due either to an alteration in the effectiveness of the oestrus hormone, or to a lowered renal threshold that permits its rapid elimination from the blood stream. It has been amply demonstrated however, that during the latter half of pregnancy in the human, there are far larger quantities of oestrin in both the blood and urine than at other times (Ascheim and Zondek, 1927; Laquer, 1927; Slotta, 1927; Veler and Doisy, 1928). This can only mean that the human uterus is quiescent in spite of a great concentration of oestrin in the blood, if the human uterus behaves as does the uterus of the rabbit in this respect. From these considerations, therefore, it seems likely that oestrin is rendered

ineffective during pregnancy, as regards uterine motility. This is further borne out by the observation that 5 cc. of whole human urine of pregnancy inhibit uterine motility within five to eight hours after it is injected intravenously into a doe showing marked uterine activity (Reynolds and Friedman, 1930b). This happens in spite of the fact that there is a potent oestrus producing substance in the urine plus that presumably present also in the blood of the rabbit. We have roughly estimated that the 5 cc. of urine which we injected contained oestrin in amounts about equivalent to the minimal effective quantity noted above, in the recent castrate experiments.

The literature does not afford much help on this seeming paradox, inasmuch as heretofore there has been no way to estimate the nature of the action of oestrin in non-pregnant animals. Parkes (1930b) very recently has reviewed the literature, and extended the observations on the effect of the water soluble oestrin in the course of pregnancy in experimental animals. He found that it was possible to produce abortion in eighteen out of twenty-four mice by the injection of a total of 360 m. u. (approximately 48 r. u.) of oestrin in the course of thirty-six hours. Smaller quantities, 260 m. u. (32 r. u.) would not regularly produce abortion. Moreover, Fraenkel (1927) was unable to interrupt pregnancy in the rabbit, and Loeb and Kountz (1928) report negative results in the guinea pig. The difference may be one of species difference, or what is more likely, only excessive or toxic amounts of oestrin are effective. Considered in this light, these data may support the suggestion that oestrin in the pregnant animal is rendered ineffective in its effect on the motility of the uterus. Only quantitative studies on the uterus *in vivo* will supply an answer to this question. Such experiments on the unanesthetized, pseudo-pregnant rabbit are being carried out in this laboratory at the present time.

It is a pleasure to acknowledge my indebtedness to Dr. Maurice H. Friedman, who first suggested the uterine fistula preparation and its application to this and other problems, for the many kindly criticisms and suggestions he has given me.

CONCLUSIONS

1. Marked uterine activity in the non-pregnant rabbit is associated with oestrus. Feeble activity has been twice seen in oestrus; moderate activity has been seen seven times. Anoestrus is usually associated with only feeble activity, or none at all.

2. The motility of the uterus of a rabbit deprived of its ovaries during oestrus becomes irregular and progressively less in two to four days. Complete quiescence eventually supervenes. The change has been

demonstrated in the same rabbit, by obtaining records both before and after castration.

3. Replacement therapy in castrate rabbits with Theelin and Estrogen, commercial water and lipoid soluble oestrogenic extracts, may impart to the quiescent uterus of those animals, activity such as usually occurs in the presence of the ovaries during oestrus.

4. Because of the conclusions noted in 1, 2 and 3 above, we feel justified in concluding that the ovary of the non-pregnant rabbit normally exerts a profound effect upon the motility of the uterus.

5. Due to the occasional irregularities in uterine motility seen during oestrus, mentioned above, it appears that certain unknown extra-ovarian influences sometimes, though rarely, modify or completely annul the effect of the ovary on the motility of the uterus.

6. The minimal effective dose of Theelin administered as we have used it, is less than 2 rat units per kilogram bodyweight in recently castrated rabbits. Twenty rat units per kilo is the minimal effective dose in rabbits ovariectomized for three weeks.

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